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(54) Staphylococcus aureus polynucleotides and sequences

(57) The present invention provides polynucleotide sequences of the genome of *Staphylococcus aureus*, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

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Description

The present invention relates to the field of molecular biology. In particular, it relates to, among other things, nucleotide sequences of *Staphylococcus aureus,* contigs, ORFs, fragments, probes, primers and related polynucleotides thereof, peptides and polypeptides encoded by the sequences, and uses of the polynucleotides and sequences thereof, such as in fermentation, polypeptide production, assays and pharmaceutical development, among others.

The genus *Staphylococcus* includes at least 20 distinct species. (For a review see Novick, R. P., The *Staphylococcus* as a Molecular Genetic System, Chapter 1, pgs. 1-37 in MOLECULAR BIOLOGY OF THE *STAPHYLOCOCCI,* R. Novick, Ed., VCH Publishers, New York (1990)). Species differ from one another by 80% or more, by hybridization kinetics, whereas strains within a species are at least 90% identical by the same measure.

The species *Staphylococcus aureus,* a gram-positive, facultatively aerobic, clump-forming cocci, is among the most important etiological agents of bacterial infection in humans, as discussed briefly below.

Human Health and *S. Aureus*

Staphylococcus aureus is a ubiquitous pathogen. (See, for instance, Mims *et al.,* MEDICAL MICROBIOLOGY, Mosby-Year Book Europe Limited, London, UK (1993)). It is an etiological agent of a variety of conditions, ranging in severity from mild to fatal. A few of the more common conditions caused by *S. aureus* infection are burns, cellulitis, eyelid infections, food poisoning, joint infections, neonatal conjunctivitis,osteomyelitis, skin infections, surgical wound infection, scalded skin syndrome and toxic shock syndrome, some of which are described further below.

Burns

Burn wounds generally are sterile initially. However, they generally compromise physical and immune barriers to infection, cause loss of fluid and electrolytes and result in local or general physiological dysfunction. After cooling, contact with viable bacteria results in mixed colonization at the injury site. Infection may be restricted to the non-viable debris on the burn surface ("eschar"), it may progress into full skin infection and invade viable tissue below the eschar and it may reach below the skin, enter the lymphatic and blood circulation and develop into septicaemia. *S. aureus* is among the most important pathogens typically found in burn wound infections. It can destroy granulation tissue and produce severe septicaemia.

Cellulitis

Cellulitis, an acute infection of the skin that expands from a typically superficial origin to spread below the cutaneous layer, most commonly is caused by *S. aureus* in conjunction with *S. pyrogenes*. Cellulitis can lead to systemic infection. In fact, cellulitis can be one aspect of synergistic bacterial gangrene. This condition typically is caused by a mixture of *S. aureus* and microaerophilic *streptococci.* It causes necrosis and treatment is limited to excision of the necrotic tissue. The condition often is fatal.

Eyelid infections

S. aureus is the cause of styes and of sticky eye" in neonates, among other eye infections. Typically such infections are limited to the surface of the eye, and may occasionally penetrate the surface with more severe consequences.

Food poisoning

Some strains of *S. aureus* produce one or more of five serologically distinct, heat and acid stable enterotoxins that are not destroyed by digestive process of the stomach and small intestine (enterotoxins A-E). Ingestion of the toxin, in sufficient quantities, typically results in severe vomiting, but not diarrhoea. The effect does not require viable bacteria. Although the toxins are known, their mechanism of action is not understood.

Joint infections

S. aureus infects bone joints causing diseases such osteomyelitis.

Osteomyelitis

S. aureus is the most common causative agent of haematogenous osteomyelitis. The disease tends to occur in

children and adolescents more than adults and it is associated with non-penetrating injuries to bones. Infection typically occurs in the long end of growing bone, hence its occurrence in physically immature populations. Most often, infection is localized in the vicinity of sprouting capillary loops adjacent to epiphysial growth plates in the end of long, growing bones.

Skin infections

S. aureus is the most common pathogen of such minor skin infections as abscesses and boils. Such infections often are resolved by normal host response mechanisms, but they also can develop into severe internal infections. Recurrent infections of the nasal passages plague nasal carriers of *S. aureus*.

Surgical Wound Infections

Surgical wounds often penetrate far into the body. Infection of such wound thus poses a grave risk to the patient. S. aureus is the most important causative agent of infections in surgical wounds. *S. aureus* is unusually adept at invading surgical wounds; sutured wounds can be infected by far fewer *S. aureus* cells then are necessary to cause infection in normal skin. Invasion of surgical wound can lead to severe *S. aureus* septicaemia. Invasion of the blood stream by *S. aureus* can lead to seeding and infection of internal organs, particularly heart valves and bone, causing systemic diseases, such as endocarditis and osteomyelitis.

Scalded Skin Syndrome

S. aureus is responsible for "scalded skin syndrome" (also called toxic epidermal necrosis, Ritter's disease and Lyell's disease). This diseases occurs in older children, typically in outbreaks caused by flowering of *S. aureus* strains produce exfoliation(also called scalded skin syndrome toxin). Although the bacteria initially may infect only a minor lesion, the toxin destroys intercellular connections, spreads epidermal layers and allows the infection to penetrate the outer layer of the skin, producing the desquamation that typifies the diseases. Shedding of the outer layer of skin generally reveals normal skin below, but fluid lost in the process can produce severe injury in young children if it is not treated properly.

Toxic Shock Syndrome

Toxic shock syndrome is caused by strains of *S. aureus* that produce the so-called toxic shock syndrome toxin. The disease can be caused by *S. aureus* infection at any site, but it is too often erroneously viewed exclusively as a disease solely of women who use tampons. The disease involves toxaemia and septicaemia, and can be fatal.

Nocosomial Infections

In the 1984 National Nocosomial Infection Surveillance Study ("NNIS") *S. aureus* was the most prevalent agent of surgical wound infections in many hospital services, including medicine, surgery, obstetrics, pediatrics and newborns.

Resistance to drugs of S. aureus strains

Prior to the introduction of penicillin the prognosis for patients seriously infected with *S. aureus* was unfavorable. Following the introduction of penicillin in the early 1940s even the worst *S. aureus* infections generally could be treated successfully. The emergence of penicillin-resistant strains of *S. aureus* did not take long, however. Most strains of *S. aureus* encountered in hospital infections today do not respond to penicillin; although, fortunately, this is not the case for *S. aureus* encountered in community infections.

It is well known now that penicillin-resistant strains of *S. aureus* produce a lactamase which converts penicillin to pencillinoic acid, and thereby destroys antibiotic activity. Furthermore, the lactamase gene often is propagated episomally, typically on a plasmid, and often is only one of several genes on an episomal element that, together, confer multidrug resistance.

Methicillins, introduced in the 1960s, largely overcame the problem of penicillin resistance in *S. aureus*. These compounds conserve the portions of penicillin responsible for antibiotic activity and modify or alter other portions that make penicillin a good substrate for inactivating lactamases. However, methicillin resistance has emerged in *S. aureus,* along with resistance to many other antibiotics effective against this organism, including aminoglycosides, tetracycline, chloramphenicol, macrolides and lincosamides. In fact, methicillin-resistant strains of *S. aureus* generally are multiply drug resistant.

The molecular genetics of most types of drug resistance in *S. aureus* has been elucidated (See Lyon *et al., Microbiology Reviews* 51: 88-134 (1987)). Generally, resistance is mediated by plasmids, as noted above regarding penicillin resistance; however, several stable forms of drug resistance have been observed that apparently involve integration of a resistance element into the *S. aureus* genome itself.

Thus far each new antibiotic gives rise to resistance strains, stains emerge that are resistance to multiple drugs and increasingly persistent forms of resistance begin to emerge. Drug resistance of *S. aureus* infections already poses significant treatment difficulties, which are likely to get much worse unless new therapeutic agents are developed.

Molecular Genetics *of Staphylococcus Aureus*

Despite its importance in, among other things, human disease, relatively little is known about the genome of this organism.

Most genetic studies of *S. aureus* have been carried out using the the strain NCTC8325, which contains prophages psi11 psi12 and psi13, and the UV-cured derivative of this strain, 8325-4 (also referred to as RN450), which is free of the prophages.

These studies revealed that the *S. aureus* genome, like that of other *staphylococci,* consists of one circular, covalently closed, double-stranded DNA and a collection of so-called variable accessory genetic elements, such as prophages, plasmids, transposons and the like.

Physical characterization of the genome has not been carried out in any detail. Pattee *et al.* published a low resolution and incomplete genetic and physical map of the chromosome of *S. aureus* strain NCTC 8325. (Pattee *et al.* Genetic and Physical Mapping of Chromosome of *Staphylococcus aureus* NCTC 8325, Chapter 11, pgs. 163-169 in. MOLECULAR BIOLOGY OF THE *STAPHYLOCOCCI,* R.P. Novick, Ed., VCH Publishers, New York, (1990) The genetic map largely was produced by mapping insertions of Tn551 and Tn4001, which, respectively, confer erythromycin and gentamicin resistance, and by analysis of SmaI-digested DNA by Pulsed Field Gel Electrophoresis ("PFGE").

The map was of low resolution; even estimating the physical size of the genome was difficult, according to the investigators. The size of the largest SmaI chromosome fragment, for instance, was too large for accurate sizing by PFGE. To estimate its size, additional restriction sites had to be introduced into the chromosome using a transposon containing a SmaI recognition sequence.

In sum, most physical characteristics and almost all of the genes of *Staphylococcus aureus* are unknown. Among the few genes that have been identified, most have not been physically mapped or characterized in detail. Only a very few genes of this organism have been sequenced. (See, for instance Thornsberry, J. , *Antimicrobial Chemotherapy* 21 Suppl C: 9-16 (1988), current versions of GENBANK and other nucleic acid databases, and references that relate to the genome of *S. aureus* such as those set out elsewhere herein.)

It is clear that the etiology of diseases mediated or exacerbated by *S. aureus* infection involves the programmed expression of *S. aureus* genes, and that characterizing the genes and their patterns of expression would add dramatically to our understanding of the organism and its host interactions. Knowledge of *S. aureus* genes and genomic organization would dramatically improve understanding of disease etiology and lead to improved and new ways of preventing, ameliorating, arresting and reversing diseases. Moreover, characterized genes and genomic fragments of *S. aureus* would provide reagents for, among other things, detecting, characterizing and controlling *S. aureus* infections. There is a need therefore to characterize the genome of *S. aureus* and for polynucleotides and sequences of this organism.

The present invention is based on the sequencing of fragments of the *Staphylococcus aureus* genome. The primary nucleotide sequences which were generated are provided in SEQ ID NOS: 1-5,191.

The present invention provides the nucleotide sequence of several thousand contigs of the *Staphylococcus aureus* genome, which are listed in tables below and set out in the Sequence Listing submitted herewith, and representative fragments thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan. In one embodiment, the present invention is provided as contiguous strings of primary sequence information corresponding to the nucleotide sequences depicted in SEQ ID NOS:1-5,191.

The present invention further provides nucleotide sequences which are at least 95%, preferably 99% and most preferably 99.9%, identical to the nucleotide sequences of SEQ ID NOS:1-5,191.

The nucleotide sequence of SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence which is at least 95%, preferably 99% and most preferably 99.9%, identical to the nucleotide sequence of SEQ ID NOS:1-5,191 may be provided in a variety of mediums to facilitate its use. In one application of this embodiment, the sequences of the present invention are recorded on computer readable media. Such media includes, but is not limited to:magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media.

The present invention further provides systems, particularly computer-based systems which contain the sequence

information herein described stored in a data storage means. Such systems are designed to identify commercially important fragments of the *Staphylococcus aureus* genome.

Another embodiment of the present invention is directed to fragments, preferably isolated fragments, of the *Staphylococcus aureus* genome having particular structural or functional attributes. Such fragments of the *Staphylococcus aureus* genome of the present invention include, but are not limited to, fragments which encode peptides, hereinafter referred to as open reading frames or ORFs," fragments which modulate the expression of an operably linked ORF, hereinafter referred to as expression modulating fragments or EMFs," and fragments which can be used to diagnose the presence of *Staphylococcus aureus* in a sample, hereinafter referred to as diagnostic fragments or "DFs."

Each of the ORFs in fragments of the *Staphylococcus aureus* genome disclosed in Tables 1-3, and the EMFs found 5' to the ORFs, can be used in numerous ways as polynucleotide reagents. For instance, the sequences can be used as diagnostic probes or amplification primers for detecting or determining the presence of a specific microbe in a sample, to selectively control gene expression in a host and in the production of polypeptides, such as polypeptides encoded by ORFs of the present invention, particular those polypeptides that have a pharmacological activity.

The present invention further includes recombinant constructs comprising one or more fragments of the *Staphylococcus aureus* genome of the present invention. The recombinant constructs of the present invention comprise vectors, such as a plasmid or viral vector, into which a fragment of the *Staphylococcus aureus* has been inserted.

The present invention further provides host cells containing any of the isolated fragments of the *Staphylococcus aureus* genome of the present invention. The host cells can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic cell, such as a yeast cell, or a procaryotic cell such as a bacterial cell.

The present invention is further directed to polypeptides and proteins, preferably isolated polypeptides and proteins, encoded by ORFs of the present invention. A variety of methods, well known to those of skill in the art, routinely may be utilized to obtain any of the polypeptides and proteins of the present invention. For instance, polypeptides and proteins of the present invention having relatively short, simple amino acid sequences readily can be synthesized using commercially available automated peptide synthesizers. Polypeptides and proteins of the present invention also may be purified from bacterial cells which naturally produce the protein. Yet another alternative is to purify polypeptide and proteins of the present invention can from cells which have been altered to express them.

The invention further provides polypeptides, preferably isolated polypeptides, comprising *Staphylococcus aureus* epitopes and vaccine compositions comprising such polypeptides. Also provided are methods for vacciniating an individual against *Staphylococcus aureus* infection.

The invention further provides methods of obtaining homologs of the fragments of the *Staphylococcus aureus* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. Specifically, by using the nucleotide and amino acid sequences disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

The invention further provides antibodies which selectively bind polypeptides and proteins of the present invention. Such antibodies include both monoclonal and polyclonal antibodies.

The invention further provides hybridomas which produce the above-described antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

The present invention further provides methods of identifying test samples derived from cells which express one of the ORFs of the present invention, or a homolog thereof. Such methods comprise incubating a test sample with one or more of the antibodies of the present invention, or one or more of the Dfs or antigens of the present invention, under conditions which allow a skilled artisan to determine if the sample contains the ORF or product produced therefrom.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the above-described assays.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the antibodies, antigens, or one of the DFs of the present invention; and (b) one or more other containers comprising one or more of the following:wash reagents, reagents capable of detecting presence of bound antibodies, antigens or hybridized DFs.

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents capable of binding to a polypeptide or protein encoded by one of the ORFs of the present invention. Specifically, such agents include, as further described below, antibodies, peptides, carbohydrates, pharmaceutical agents and the like. Such methods comprise steps of: (a)contacting an agent with an isolated protein encoded by one of the ORFs of the present invention; and (b)determining whether the agent binds to said protein.

The present genomic sequences of *Staphylococcus aureus* will be of great value to all laboratories working with this organism and for a variety of commercial purposes. Many fragments of the *Staphylococcus aureus* genome will be immediately identified by similarity searches against GenBank or protein databases and will be of immediate value to *Staphylococcus aureus* researchers and for immediate commercial value for the production of proteins or to control gene expression.

The methodology and technology for elucidating extensive genomic sequences of bacterial and other genomes

has and will greatly enhance the ability to analyze and understand chromosomal organization. In particular, sequenced contigs and genomes will provide the models for developing tools for the analysis of chromosome structure and function, including the ability to identify genes within large segments of genomic DNA, the structure, position, and spacing of regulatory elements, the identification of genes with potential industrial applications, and the ability to do comparative genomic and molecular phylogeny.

FIGURE 1 is a block diagram of a computer system (102) that can be used to implement computer-based systems of present invention.

FIGURE 2 is a schematic diagram depicting the data flow and computer programs used to collect, assemble, edit and annotate the contigs of the *Staphylococcus aureus* genome of the present invention. Both Macintosh and Unix platforms are used to handle the AB 373 and 377 sequence data files, largely as described in Kerlavage *et al., Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences,* 585, IEEE Computer Society Press, Washington D.C. (1993). Factura (AB) is a Macintosh program designed for automatic vector sequence removal and end-trimming of sequence files. The program Loadis runs on a Macintosh platform and parses the feature data extracted from the sequence files by Factura to the Unix based *Staphylococcus aureus* relational database. Assembly of contigs (and whole genome sequences) is accomplished by retrieving a specific set of sequence files and their associated features using extrseq, a Unix utility for retrieving sequences from an SQL database. The resulting sequence file is processed by seq_filter to trim portions of the sequences with more than 2% ambiguous nucleotides. The sequence files were assembled using TIGR Assembler, an assembly engine designed at The Institute for Genomic Research (TIGR") for rapid and accurate assembly of thousands of sequence fragments. The collection of contigs generated by the assembly step is loaded into the database with the lassie program. Identification of open reading frames (ORFs) is accomplished by processing contigs with zorf. The ORFs are searched against *S. aureus* sequences from Genbank and against all protein sequences using the BLASTN and BLASTP programs, described in Altschul *et al., J. Mol. Biol.* 215: 403-410 (1990)). Results of the ORF determination and similarity searching steps were loaded into the database. As described below, some results of the determination and the searches are set out in Tables 1-3..

The present invention is based on the sequencing of fragments of the *Staphylococcus aureus* genome and analysis of the sequences. The primary nucleotide sequences generated by sequencing the fragments are provided in SEQ ID NOS:1-5,191. (As used herein, the "primary sequence" refers to the nucleotide sequence represented by the IUPAC nomenclature system.)

In addition to the aforementioned *Staphylococcus aureus* polynucleotide and polynucleotide sequences, the present invention provides the nucleotide sequences of SEQ ID NOS:1-5,191, or representative fragments thereof, in a form which can be readily used, analyzed, and interpreted by a skilled artisan.

As used herein, a "representative fragment of the nucleotide sequence depicted in SEQ ID NOS:1-5,191" refers to any portion of the SEQ ID NOS:1-5,191 which is not presently represented within a publicly available database. Preferred representative fragments of the present invention are *Staphylococcus aureus* open reading frames (ORFs"), expression modulating fragment (EMFs") and fragments which can be used to diagnose the presence of *Staphylococcus aureus* in sample ("DFs"). A non-limiting identification of preferred representative fragments is provided in Tables 1-3.

As discussed in detail below, the information provided in SEQ ID NOS:1-5,191 and in Tables 1-3 together with routine cloning, synthesis, sequencing and assay methods will enable those skilled in the art to clone and sequence all "representative fragments" of interest, including open reading frames encoding a large variety of *Staphylococcus aureus* proteins.

While the presently disclosed sequences of SEQ ID NOS:1-5,191 are highly accurate, sequencing techniques are not perfect and, in relatively rare instances, further investigation of a fragment or sequence of the invention may reveal a nucleotide sequence error present in a nucleotide sequence disclosed in SEQ ID NOS:1-5,191. However, once the present invention is made available (*i.e.,* once the information in SEQ ID NOS:1-5,191 and Tables 1-3 has been made available), resolving a rare sequencing error in SEQ ID NOS:1-5,191 will be well within the skill of the art. The present disclosure makes available sufficient sequence information to allow any of the described contigs or portions thereof to be obtained readily by straightforward application of routine techniques. Further sequencing of such polynucleotide may proceed in like manner using manual and automated sequencing methods which are employed ubiquitous in the art. Nucleotide sequence editing software is publicly available. For example, Applied Biosystem's (AB) AutoAssembler can be used as an aid during visual inspection of nucleotide sequences. By employing such routine techniques potential errors readily may be identified and the correct sequence then may be ascertained by targeting further sequencing effort, also of a routine nature, to the region containing the potential error.

Even if all of the very rare sequencing errors in SEQ ID NOS:1-5,191 were corrected, the resulting nucleotide sequences would still be at least 95% identical, nearly all would be at least 99% identical, and the great majority would be at least 99.9% identical to the nucleotide sequences of SEQ ID NOS:1-5,191.

As discussed elsewhere hererin, polynucleotides of the present invention readily may be obtained by routine application of well known and standard procedures for cloning and sequencing DNA. Detailed methods for obtaining

libraries and for sequencing are provided below, for instance. A wide variety of *Staphylococcus aureus* strains that can be used to prepare *S aureus* genomic DNA for cloning and for obtaining polynucleotides of the present invention are available to the public from recognized depository institutions, such as the American Type Culture Collection (ATCC").

The nucleotide sequences of the genomes from different strains of *Staphylococcus aureus* differ somewhat. However, the nucleotide sequences of the genomes of all *Staphylococcus aureus* strains will be at least 95% identical, in corresponding part, to the nucleotide sequences provided in SEQ ID NOS:1-5,191. Nearly all will be at least 99% identical and the great majority will be 99.9% identical.

Thus, the present invention further provides nucleotide sequences which are at least 95%, preferably 99% and most preferably 99.9% identical to the nucleotide sequences of SEQ ID NOS:1-5,191, in a form which can be readily used, analyzed and interpreted by the skilled artisan.

Methods for determining whether a nucleotide sequence is at least 95%, at least 99% or at least 99.9% identical to the nucleotide sequences of SEQ ID NOS:1-5,191 are routine and readily available to the skilled artisan. For example, the well known fasta algorithm described in Pearson and Lipman, *Proc. Natl. Acad. Sci. USA* 85: 2444 (1988) can be used to generate the percent identity of nucleotide sequences. The BLASTN program also can be used to generate an identity score of polynucleotides compared to one another.

COMPUTER RELATED EMBODIMENTS

The nucleotide sequences provided in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a polynucleotide sequence of SEQ ID NOS:1-5,191 may be "provided" in a variety of mediums to facilitate use thereof. As used herein, Òprovided" refers to a manufacture, other than an isolated nucleic acid molecule, which contains a nucleotide sequence of the present invention; *i.e.,* a nucleotide sequence provided in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a polynucleotide of SEQ ID NOS:1-5,191. Such a manufacture provides a large portion of the *Staphylococcus aureus* genome and parts thereof (*e.g.,* a *Staphylococcus aureus* open reading frame (ORF)) in a form which allows a skilled artisan to examine the manufacture using means not directly applicable to examining the *Staphylococcus aureus* genome or a subset thereof as it exists in nature or in purified form.

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD- ROM; electrical storage media such as RAM and ROM; and hybrids of these categories, such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. Likewise, it will be clear to those of skill how additional computer readable media that may be developed also can be used to create analogous manufactures having recorded thereon a nucleotide sequence of the present invention.

As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently know methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially- available software such as WordPerfect and MicroSoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data-processor structuring formats (*e.g.,* text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. Thus, by providing in computer readable form the nucleotide sequences of SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably at least 99% and most preferably at least 99.9% identical to a sequence of SEQ ID NOS:1-5,191 the present invention enables the skilled artisan routinely to access the provided sequence information for a wide variety of purposes.

The examples which follow demonstrate how software which implements the BLAST (Altschul *et al.,* J. Mol. Biol. 215:403410 (1990)) and BLAZE (Brutlag *et al.,* Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system was used to identify open reading frames (ORFs) within the *Staphylococcus aureus* genome which contain homology to ORFs or proteins from both *Staphylococcus aureus* and from other organisms. Among the ORFs discussed

herein are protein encoding fragments of the *Staphylococcus aureus* genome useful in producing commercially important proteins, such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

The present invention further provides systems, particularly computer-based systems, which contain the sequence information described herein. Such systems are designed to identify, among other things, commercially important fragments of the *Staphylococcus aureus* genome.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.

As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means.

As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer- based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of the present genomic sequences which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, MacPattern (EMBL), BLASTN and BLASTX (NCBIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems.

As used herein, a "target sequence" can be any DNA or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 100 amino acids or from about 30 to 300 nucleotide residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzymic active sites and signal sequences. Nucleic acid target motifs include, but are not limited to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

A variety of structural formats for the input and output means can be used to input and output the information in the computer-based systems of the present invention. A preferred format for an output means ranks fragments of the *Staphylococcus aureus* genomic sequences possessing varying degrees of homology to the target sequence or target motif. Such presentation provides a skilled artisan with a ranking of sequences which contain various amounts of the target sequence or target motif and identifies the degree of homology contained in the identified fragment.

A variety of comparing means can be used to compare a target sequence or target motif with the data storage means to identify sequence fragments of the *Staphylococcus aureus* genome. In the present examples, implementing software which implement the BLAST and BLAZE algorithms, described in Altschul *et al., J. Mol. Biol.* 215: 403-410 (1990), was used to identify open reading frames within the *Staphylococcus aureus* genome. A skilled artisan can readily recognize that any one of the publicly available homology search programs can be used as the search means for the computer-based systems of the present invention. Of course, suitable proprietary systems that may be known to those of skill also may be employed in this regard.

Figure 1 provides a block diagram of a computer system illustrative of embodiments of this aspect of present invention. The computer system 102 includes a processor 106 connected to a bus 104. Also connected to the bus 104 are a main memory 108 (preferably implemented as random access memory, RAM) and a variety of secondary storage devices 110, such as a hard drive 112 and a removable medium storage device 114. The removable medium storage device 114 may represent, for example, a floppy disk drive, a CD-ROM drive, a magnetic tape drive, *etc.* A removable storage medium 116 (such as a floppy disk, a compact disk, a magnetic tape, *etc.*) containing control logic and/or data recorded therein may be inserted into the removable medium storage device 114. The computer system 102 includes appropriate software for reading the control logic and/or the data from the removable medium storage device 114, once it is inserted into the removable medium storage device 114.

A nucleotide sequence of the present invention may be stored in a well known manner in the main memory 108, any of the secondary storage devices 110, and/or a removable storage medium 116. During execution, software for

accessing and processing the genomic sequence (such as search tools, comparing tools, *etc.*) reside in main memory 108, in accordance with the requirements and operating parameters of the operating system, the hardware system and the software program or programs.

BIOCHEMICAL EMBODIMENTS

Other embodiments of the present invention are directed to fragments of the *Staphylococcus aureus* genome, preferably to isolated fragments. The fragments of the *Staphylococcus aureus* genome of the present invention include, but are not limited to fragments which encode peptides, hereinafter open reading frames (ORFs), fragments which modulate the expression of an operably linked ORF, hereinafter expression modulating fragments (EMFs) and fragments which can be used to diagnose the presence of *Staphylococcus aureus* in a sample, hereinafter diagnostic fragments (DFs).

As used herein, an "isolated nucleic acid molecule" or an "isolated fragment of the *Staphylococcus aureus* genome" refers to a nucleic acid molecule possessing a specific nucleotide sequence which has been subjected to purification means to reduce, from the composition, the number of compounds which are normally associated with the composition. Particularly, the term refers to the nucleic acid molecules having the sequences set out in SEQ ID NOS:1-5,191, to representative fragments thereof as described above, to polynucleotides at least 95%, preferably at least 99% and especially preferably at least 99.9% identical in sequence thereto, also as set out above.

A variety of purification means can be used to generated the isolated fragments of the present invention. These include, but are not limited to methods which separate constituents of a solution based on charge, solubility, or size.

In one embodiment, *Staphylococcus aureus* DNA can be mechanically sheared to produce fragments of 15-20 kb in length. These fragments can then be used to generate an *Staphylococcus aureus* library by inserting them into lambda clones as described in the Examples below. Primers flanking, for example, an ORF, such as those enumerated in Tables 1-3 can then be generated using nucleotide sequence information provided in SEQ ID NOS: 1-5,191. Well known and routine techniques of PCR cloning then can be used to isolate the ORF from the lambda DNA library of Staphylococcus aureus genomic DNA. Thus, given the availability of SEQ ID NOS:1-5,191, the information in Tables 1, 2 and 3, and the information that may be obtained readily by analysis of the sequences of SEQ ID NOS:1-5,191 using methods set out above, those of skill will be enabled by the present disclosure to isolate any ORF-containing or other nucleic acid fragment of the present invention.

The isolated nucleic acid molecules of the present invention include, but are not limited to single stranded and double stranded DNA, and single stranded RNA.

As used herein, an "open reading frame," ORF, means a series of triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

Tables 1, 2 and 3 list ORFs in the *Staphylococcus aureus* genomic contigs of the present invention that were identified as putative coding regions by the GeneMark software using organism-specific second-order Markov probability transition matrices. It will be appreciated that other criteria can be used, in accordance with well known analytical methods, such as those discussed herein, to generate more inclusive, more restrictive or more selective lists.

Table 1 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that are at least 80 amino acids long and over a continuous region of at least 50 bases which are 95% or more identical (by BLAST analysis) to an *S. aureus* nucleotide sequence available through Genbank in November 1996.

Table 2 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that are not in Table 1 and match, with a BLASTP probability score of 0.01 or less, a polypeptide sequence available through Genbank by September 1996.

Table 3 sets out ORFs in the *Staphylococcus aureus* contigs of the present invention that do not match significantly, by BLASTP analysis, a polypeptide sequence available through Genbank by September 1996.

In each table, the first and second columns identify the ORF by, respectively, contig number and ORF number within the contig; the third column indicates the reading frame, taking the first 5' nucleotide of the contig as the start of the +1 frame; the fourth column indicates the first nucleotide of the ORF, counting from the 5' end of the contig strand; and the fifth column indicates the length of each ORF in nucleotides.

In Tables 1 and 2, column six, lists the Reference" for the closest matching sequence available through Genbank. These reference numbers are the databases entry numbers commonly used by those of skill in the art, who will be familiar with their denominators. Descriptions of the numenclature are available from the National Center for Biotechnology Information. Column seven in Tables 1 and 2 provides the gene name" of the matching sequence; column eight provides the BLAST identity" score from the comparison of the ORF and the homologous gene; and column nine indicates the length in nucleotides of the highest scoring segment pair" identified by the BLAST identity analysis.

In Table 3, the last column, column six, indicates the length of each ORF in amino acid residues.

The concepts of percent identity and percent similarity of two polypeptide sequences is well understood in the art. For example, two polypeptides 10 amino acids in length which differ at three amino acid positions (*e.g.*, at positions

1, 3 and 5) are said to have a percent identity of 70%. However, the same two polypeptides would be deemed to have a percent similarity of 80% if, for example at position 5, the amino acids moieties, although not identical, were "similar" (*i.e.*, possessed similar biochemical characteristics). Many programs for analysis of nucleotide or amino acid sequence similarity, such as fasta and BLAST specifically list per cent identity of a matching region as an output parameter. Thus, for instance, Tables 1 and 2 herein enumerate the per cent identity" of the highest scoring segment pair" in each ORF and its listed relative. Further details concerning the algorithms and criteria used for homology searches are provided below and are described in the pertinent literature highlighted by the citations provided below.

It will be appreciated that other criteria can be used to generate more inclusive and more exclusive listings of the types set out in the tables. As those of skill will appreciate, narrow and broad searches both are useful. Thus, a skilled artisan can readily identify ORFs in contigs of the *Staphylococcus aureus* genome other than those listed in Tables 1-3, such as ORFs which are overlapping or encoded by the opposite strand of an identified ORF in addition to those ascertainable using the computer-based systems of the present invention.

As used herein, an "expression modulating fragment," EMF, means a series of nucleotide molecules which modulates the expression of an operably linked ORF or EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are fragments which induce the expression or an operably linked ORF in response to a specific regulatory factor or physiological event.

EMF sequences can be identified within the contigs of the *Staphylococcus aureus* genome by their proximity to the ORFs provided in Tables 1-3. An intergenic segment, or a fragment of the intergenic segment, from about 10 to 200 nucleotides in length, taken from any one of the ORFs of Tables 1-3 will modulate the expression of an operably linked ORF in a fashion similar to that found with the naturally linked ORF sequence. As used herein, an "intergenic segment" refers to fragments of the *Staphylococcus aureus* genome which are between two ORF(s) herein described. EMFs also can be identified using known EMFs as a target sequence or target motif in the computer-based systems of the present invention. Further, the two methods can be combined and used together.

The presence and activity of an EMF can be confirmed using an EMF trap vector. An EMF trap vector contains a cloning site linked to a marker sequence. A marker sequence encodes an identifiable phenotype, such as antibiotic resistance or a complementing nutrition auxotrophic factor, which can be identified or assayed when the EMF trap vector is placed within an appropriate host under appropriate conditions. As described above, a EMF will modulate the expression of an operably linked marker sequence. A more detailed discussion of various marker sequences is provided below.

A sequence which is suspected as being an EMF is cloned in all three reading frames in one or more restriction sites upstream from the marker sequence in the EMF trap vector. The vector is then transformed into an appropriate host using known procedures and the phenotype of the transformed host in examined under appropriate conditions. As described above, an EMF will modulate the expression of an operably linked marker sequence.

As used herein, a "diagnostic fragment," DF, means a series of nucleotide molecules which selectively hybridize to *Staphylococcus aureus* sequences. DFs can be readily identified by identifying unique sequences within contigs of the *Staphylococcus aureus* genome, such as by using well-known computer analysis software, and by generating and testing probes or amplification primers consisting of the DF sequence in an appropriate diagnostic format which determines amplification or hybridization selectivity.

The sequences falling within the scope of the present invention are not limited to the specific sequences herein described, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequences provided in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95%, preferably 99% and most preferably 99.9% identical to SEQ ID NOS:1-5,191, with a sequence from another isolate of the same species.

Furthermore, to accomodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the nucleic acid sequences mentioned above. In other words, in the coding region of an ORF, substitution of one codon for another which encodes the same amino acid is expressly contemplated.

Any specific sequence disclosed herein can be readily screened for errors by resequencing a particular fragment, such as an ORF, in both directions (*i.e.*, sequence both strands). Alternatively, error screening can be performed by sequencing corresponding polynucleotides of *Staphylococcus aureus* origin isolated by using part or all of the fragments in question as a probe or primer.

Each of the ORFs of the *Staphylococcus aureus* genome disclosed in Tables 1, 2 and 3, and the EMFs found 5' to the ORFs, can be used as polynucleotide reagents in numerous ways. For example, the sequences can be used as diagnostic probes or diagnostic amplification primers to detect the presence of a specific microbe in a sample, particular *Staphylococcus aureus*. Especially preferred in this regard are ORF such as those of Table 3, which do not match previously characterized sequences from other organisms and thus are most likely to be highly selective for *Staphylococcus aureus*. Also particularly preferred are ORFs that can be used to distinguish between strains of *Sta-*

phylococcus aureus, particularly those that distinguish medically important strain, such as drug-resistant strains.

In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA. Triple helix- formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Information from the sequences of the present invention can be used to design antisense and triple helix-forming oligonucleotides. Polynucleotides suitable for use in these methods are usually 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription, for triple-helix formation, or to the mRNA itself, for antisense inhibition. Both techniques have been demonstrated to be effective in model systems, and the requisite techniques are well known and involve routine procedures. Triple helix techniques are discussed in, for example, Lee *et al., Nucl. Acids Res.* 6: 3073 (1979); Cooney *et al., Science* 241: 456 (1988); and Dervan *et al., Science* 251: 1360 (1991). Antisense techniques in general are discussed in, for instance, Okano, *J. Neurochem.* 56: 560 (1991) and OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION, CRC Press, Boca Raton, FL (1988)).

The present invention further provides recombinant constructs comprising one or more fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention. Certain preferred recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a fragment of the *Staphylococcus aureus* genome has been inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. For vectors comprising the EMFs of the present invention, the vector may further comprise a marker sequence or heterologous ORF operably linked to the EMF.

Large numbers of suitable vectors and promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Useful bacterial vectors include phagescript, PsiX174, pBluescript SK and KS (+ and -), pNH8a, pNH16a, pNH18a, pNH46a (available from Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (available from Pharmacia). Useful eukaryotic vectors include pWLneo, pSV2cat, pOG44, pXT1, pSG (available from Stratagene) pSVK3, pBPV, pMSG, pSVL (available from Pharmacia).

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein- I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

The present invention further provides host cells containing any one of the isolated fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention, wherein the fragment has been introduced into the host cell using known methods. The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or a procaryotic cell, such as a bacterial cell.

A polynucleotide of the present invention, such as a recombinant construct comprising an ORF of the present invention, may be introduced into the host by a variety of well established techniques that are standard in the art, such as calcium phosphate transfection, DEAE, dextran mediated transfection and electroporation, which are described in, for instance, Davis, L. *et al.,* BASIC METHODS IN MOLECULAR BIOLOGY (1986).

A host cell containing one of the fragments of the *Staphylococcus aureus* genomic fragments and contigs of the present invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.,* an ORF) by nucleotide sequence but, due to the degeneracy of the Genetic Code, encode an identical polypeptide sequence.

Preferred nucleic acid fragments of the present invention are the ORFs depicted in Tables 2 and 3 which encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. This is particularly useful in producing small peptides and fragments of larger polypeptides. Such short fragments as may be obtained most readily by synthesis are useful, for example, in generating antibodies against the native polypeptide, as discussed further below.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily employ well-known methods for isolating polpeptides and proteins to isolate and purify polypeptides or proteins of the present invention produced naturally by a bacterial strain, or by other methods. Methods for isolation and purification that can be employed in this regard include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immu-

no-affinity chromatography.

The polypeptides and proteins of the present invention also can be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. Those skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, CV-1 cell, COS cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level.

"Recombinant," as used herein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (*e.g.*, yeast) expression systems. As a product, "recombinant microbial"defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g., E. coli,* will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern different from that expressed in mammalian cells.

"Nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. Generally, DNA segments encoding the polypeptides and proteins provided by this invention are assembled from fragments of the *Staphylococcus aureus* genome and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

"Recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. The expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic regulatory elements necessary for gene expression in the host, including elements required to initiate and maintain transcription at a level sufficient for suitable expression of the desired polypeptide, including, for example, promoters and, where necessary, an enhancers and a polyadenylation signal; (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate signals to initiate translation at the beginning of the desired coding region and terminate translation at its end. Structural units intended for use in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an N-terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

"Recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extra chromosomally. The cells can be prokaryotic or eukaryotic. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described in Sambrook *et al.,* MOLECULAR CLONING:A LABORATORY MANUAL, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference in its entirety.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, *e.g.*, the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), alpha-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, when desirable, provide amplification within the host.

Suitable prokaryotic hosts for transformation include strains of *Staphylococcus aureus, E. coli, B. subtilis, Salmonella typhimurium* and various species within the genera *Pseudomonas, Streptomyces,* and *Staphylococcus.* Others

may, also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (available form Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (available from Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter, where it is inducible, is derepressed or induced by appropriate means (*e.g.,* temperature shift or chemical induction) and cells are cultured for an additional period to provide for expression of the induced gene product. Thereafter cells are typically harvested, generally by centrifugation, disrupted to release expressed protein, generally by physical or chemical means, and the resulting crude extract is retained for further purification.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described in Gluzman, *Cell* 23: 175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines.

Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

Recombinant polypeptides and proteins produced in bacterial culture is usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

An additional aspect of the invention includes Staphylococcus aureus polypeptides which are useful as immunodiagnostic antigens and/or immunoprotective vaccines, collectively "immunologically useful polypeptides". Such immunologically useful polypeptides may be selected from the ORFs disclosed herein based on techniques well known in the art and described elsewhere herein. The inventors have used the following criteria to select several immunologically useful polypeptides:

As is known in the art, an amino terminal type I signal sequence directs a nascent protein across the plasma and outer membranes to the exterior of the bacterial cell. Such outermembrane polypeptides are expected to be immunologically useful. According to Izard, J. W. et al., Mol. Microbiol. 13, 765-773; (1994), polypeptides containing type I signal sequences contain the following physical attributes: The length of the type I signal sequence is approximately 15 to 25 primarily hydrophobic amino acid residues with a net positive charge in the extreme amino terminus; the central region of the signal sequence must adopt an alpha-helical conformation in a hydrophobic environment; and the region surrounding the actual site of cleavage is ideally six residues long, with small side-chain amino acids in the -1 and -3 positions.

Also known in the art is the type IV signal sequence which is an example of the several types of functional signal sequences which exist in addition to the type I signal sequence detailed above. Although functionally related, the type IV signal sequence possesses a unique set of biochemical and physical attributes (Strom, M. S. and Lory, S., J. Bacteriol. 174, 7345-7351; 1992)). These are typically six to eight amino acids with a net basic charge followed by an additional sixteen to thirty primarily hydrophobic residues. The cleavage site of a type IV signal sequence is typically after the initial six to eight amino acids at the extreme amino terminus. In addition, all type IV signal sequences contain a phenylalanine residue at the +1 site relative to the cleavage site.

Studies of the cleavage sites of twenty-six bacterial lipoprotein precursors has allowed the definition of a consensus amino acid sequence for lipoprotein cleavage. Nearly three-fourths of the bacterial lipoprotein precursors examined contained the sequence L-(A,S)-(G,A)-C at positions -3 to +1, relative to the point of cleavage (Hayashi, S. and Wu, H. C. Lipoproteins in bacteria. J Bioenerg. Biomembr. 22, 451-471; 1990).

It well known that most anchored proteins found on the surface of gram-positive bacteria possess a highly conserved carboxy terminal sequence. More than fifty such proteins from organisms such as *S. pyogenes, S. mutans, E. faecalis, S. pneumoniae,* and others, have been identified based on their extracellular location and carboxy terminal amino acid sequence (Fischetti, V. A. Gram-positive commensal bacteria deliver antigens to elicit mucosal and systemic immunity. ASM News 62, 405410; 1996). The conserved region is comprised of six charged amino acids at the extreme carboxy terminus coupled to 15-20 hydrophobic amino acids presumed to function as a transmembrane domain. Immediately adjacent to the transmembrane domain is a six amino acid sequence conserved in nearly all proteins ex-

amined. The amino acid sequence of this region is L-P-X-T-G-X, where X is any amino acid.

Amino acid sequence similarities to proteins of known function by BLAST enables the assignment of putative functions to novel amino acid sequences and allows for the selection of proteins thought to function outside the cell wall. Such proteins are well known in the art and include "lipoprotein", "periplasmic", or "antigen".

An algorithm for selecting antigenic and immunogenic *Staphylococcus aureus* polypeptides including the foregoing criteria was developed by the present inventors. Use of the algorithm by the inventors to select immunologically useful *Staphylococcus aureus* polypeptides resulted in the selection of several ORFs which are predicted to be outermembrane-associated proteins. These proteins are identified in Table 4, below, and shown in the Sequence Listing as SEQ ID NOS:5,192 to 5,255. Thus the amino acid sequence of each of several antigenic *Staphylococcus aureus* polypeptides listed in Table 4 can be determined, for example, by locating the amino acid sequence of the ORF in the Sequence Listing. Likewise the polynucleotide sequence encoding each ORF can be found by locating the corresponding polynucleotide SEQ ID in Tables 1, 2, or 3, and finding the corresponding nucleotide sequence in the sequence listing.

As will be appreciated by those of ordinary skill in the art, although a polypeptide representing an entire ORF may be the closest approximation to a protein found *in vivo*, it is not always technically practical to express a complete ORF *in vitro*. It may be very challenging to express and purify a highly hydrophobic protein by common laboratory methods. As a result, the immunologically useful polypeptides described herein as SEQ ID NOS:5,192-5,255 may have been modified slightly to simplify the production of recombinant protein, and are the preferred embodiments. In general, nucleotide sequences which encode highly hydrophobic domains, such as those found at the amino terminal signal sequence, are excluded for enhanced *in vitro* expression of the polypeptides. Furthermore, any highly hydrophobic amino acid sequences occurring at the carboxy terminus are also excluded. Such truncated polypeptides include for example the mature forms of the polypeptides expected to exist in nature.

Those of ordinary skill in the art can identify soluble portions the polypeptide identified in Table 4, and in the case of truncated polypeptides sequences shown as SEQ ID NOS:5,192-5,255, may obtain the complete predicted amino acid sequence of each polypeptide by translating the corresponding polynucleotides sequences of the corresponding ORF listed in Tables 1,2 and 3 and found in the sequence listing.

Accordingly, polypeptides comprising the complete amino acid of an immunologically useful polypeptide selected from the group of polypeptides encoded by the ORFs identified in Table 4, or an amino acid sequence at least 95% identical thereto, preferably at least 97% identical thereto, and most preferably at least 99% identical thereto form an embodiment of the invention; in addition polypeptides comprising an amino acid sequence selected from the group of amino acid sequences shown in the sequence listing as SEQ ID NOS:5,191-5,255, or an amino acid sequence at least 95% identical thereto, preferably at least 97% identical thereto and most preferably at least 99% identical thereto, form an embodiment of the invention. Polynucleotides encoding the foregoing polypeptides also form part of the present invention.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention, particularly those epitope-bearing portions (antigenic regions) identified in Table 4. The epitope-bearing portion is an immunogenic or antigenic epitope of a polypeptide of the invention. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G., Shinnick, T. M., Green, N. and Learner, R. A. (1983) "Antibodies that react with predetermined sites on proteins", Science, 219:660-666. Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals. Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson et al., Cell 37:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention. Non-limiting examples of antigenic polypeptides or peptides that can be used to generate S. aureus specific antibodies include: a polypeptide comprising peptides shown in Table 4 below. These polypeptide fragments have been determined to bear antigenic epitopes of indicated S. aureus proteins by the analysis of the Jameson-Wolf antigenic index, a representative sample of which is shown in Figure 3.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. See, e.g., Houghten, R. A. (1985) General method for the rapid solid-phase synthesis of large numbers of peptides:

specificity of antigen-antibody interaction at the level of individual amino acids. Proc. Natl. Acad. Sci. USA 82: 5131-5135; this "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,211 to Houghten et al. (1986). Epitope-bearing peptides and polypeptides of the invention are used to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow, M. et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle, F. J. et al., J. Gen. Virol. 66:2347-2354 (1985).

Immunogenic epitope-bearing peptides of the invention, i.e., those parts of a protein that elicit an antibody response when the whole protein is the immunogen, are identified according to methods known in the art. See, for instance, Geysen et al., supra. Further still, U.S. Patent No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (i.e., a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Patent No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest. Similarly, U.S. Patent No. 5,480,971 to Houghten, R. A. et al. (1996) on Peralkylated Oligopeptide Mixtures discloses linear C1-C7-alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Table 4 lists immunologically useful polypeptides identified by an algorithm which locates novel *Staphylococcus aureus* outermembrane proteins, as is described above. Also listed are epitopes or "antigenic regions" of each of the identified polypeptides. The antigenic regions, or epitopes, are delineated by two numbers x-y, where x is the number of the first amino acid in the open reading frame included within the epitope and y is the number of the last amino acid in the open reading frame included within the epitope. For example, the first epitope in ORF 168-6 is comprised of amino acids 36 to 45 of SEQ ID NO:5,192, as is described in Table 4. The inventors have identified several epitopes for each of the antigenic polypeptides identified in Table 4. Accordingly, forming part of the present invention are polypeptides comprising an amino acid sequence of one or more antigenic regions identified in Table 4. The invention further provides polynucleotides encoding such polypeptides.

The present invention further includes isolated polypeptides, proteins and nucleic acid molecules which are substantially equivalent to those herein described. As used herein, substantially equivalent can refer both to nucleic acid and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between reference and subject sequences. For purposes of the present invention, sequences having equivalent biological activity, and equivalent expression characteristics are considered substantially equivalent. For purposes of determining equivalence, truncation of the mature sequence should be disregarded.

The invention further provides methods of obtaining homologs from other strains of *Staphylococcus aureus*, of the fragments of the *Staphylococcus aureus* genome of the present invention and homologs of the proteins encoded by the ORFs of the present invention. As used herein, a sequence or protein of *Staphylococcus aureus* is defined as a homolog of a fragment of the *Staphylococcus aureus* fragments or contigs or a protein encoded by one of the ORFs of the present invention, if it shares significant homology to one of the fragments of the *Staphylococcus aureus* genome of the present invention or a protein encoded by one of the ORFs of the present invention. Specifically, by using the sequence disclosed herein as a probe or as primers, and techniques such as PCR cloning and colony/plaque hybridization, one skilled in the art can obtain homologs.

As used herein, two nucleic acid molecules or proteins are said to "share significant homology" if the two contain regions which prossess greater than 85% sequence (amino acid or nucleic acid) homology. Preferred homologs in this regard are those with more than 90% homology. Especially preferred are those with 93% or more homology. Among especially preferred homologs those with 95% or more homology are particularly preferred. Very particularly preferred among these are those with 97% and even more particularly preferred among those are homologs with 99% or more homology. The most preferred homologs among these are those with 99.9% homology or more. It will be understood that, among measures of homology, identity is particularly preferred in this regard.

Region specific primers or probes derived from the nucleotide sequence provided in SEQ ID NOS:1-5,191 or from a nucleotide sequence at least 95%, particularly at least 99%, especially at least 99.5% identical to a sequence of SEQ ID NOS:1-5,191 can be used to prime DNA synthesis and PCR amplification, as well as to identify colonies containing cloned DNA encoding a homolog. Methods suitable to this aspect of the present invention are well known and have been described in great detail in many publications such as, for example, Innis *et al.,* PCR PROTOCOLS, Academic Press, San Diego, CA (1990)).

When using primers derived from SEQ ID NOS:1-5,191 or from a nucleotide sequence having an aforementioned identity to a sequence of SEQ ID NOS:1-5,191, one skilled in the art will recognize that by employing high stringency conditions (*e.g.,* annealing at 50-60°C in 6X SSPC and 50% formamide, and washing at 50- 65°C in 0.5X SSPC) only sequences which are greater than 75% homologous to the primer will be amplified. By employing lower stringency

conditions (*e.g.,* hybridizing at 35-37°C in 5X SSPC and 40-45% formamide, and washing at 42°C in 0.5X SSPC), sequences which are greater than 40-50% homologous to the primer will also be amplified.

When using DNA probes derived from SEQ ID NOS:1-5,191, or from a nucleotide sequence having an aforementioned identity to a sequence of SEQ ID NOS:1-5,191, for colony/plaque hybridization, one skilled in the art will recognize that by employing high stringency conditions *(e.g.,* hybridizing at 50- 65°C in 5X SSPC and 50% formamide, and washing at 50- 65°C in 0.5X SSPC), sequences having regions which are greater than 90% homologous to the probe can be obtained, and that by employing lower stringency conditions (*e.g.,* hybridizing at 35-37°C in 5X SSPC and 40-45% formamide, and washing at 42°C in 0.5X SSPC), sequences having regions which are greater than 35-45% homologous to the probe will be obtained.

Any organism can be used as the source for homologs of the present invention so long as the organism naturally expresses such a protein or contains genes encoding the same. The most preferred organism for isolating homologs are bacterias which are closely related to *Staphylococcus aureus.*

ILLUSTRATIVE USES OF COMPOSITIONS OF THE INVENTION

Each ORF provided in Tables 1 and 2 is identified with a function by homology to a known gene or polypeptide. As a result, one skilled in the art can use the polypeptides of the present invention for commercial, therapeutic and industrial purposes consistent with the type of putative identification of the polypeptide. Such identifications permit one skilled in the art to use the *Staphylococcus aureus* ORFs in a manner similar to the known type of sequences for which the identification is made; for example, to ferment a particular sugar source or to produce a particular metabolite. A variety of reviews illustrative of this aspect of the invention are available, including the following reviews on the industrial use of enzymes, for example, BIOCHEMICAL ENGINEERING AND BIOTECHNOLOGY HANDBOOK, 2nd Ed., Macmillan Publications, Ltd. NY (1991) and BIOCATALYSTS IN ORGANIC SYNTHESES, Tramper *et al.,* Eds., Elsevier Science Publishers, Amsterdam, The Netherlands (1985). A variety of exemplary uses that illustrate this and similar aspects of the present invention are discussed below.

1. Biosynthetic Enzymes

Open reading frames encoding proteins involved in mediating the catalytic reactions involved in intermediary and macromolecular metabolism, the biosynthesis of small molecules, cellular processes and other functions includes enzymes involved in the degradation of the intermediary products of metabolism, enzymes involved in central intermediary metabolism, enzymes involved in respiration, both aerobic and anaerobic, enzymes involved in fermentation, enzymes involved in ATP proton motor force conversion, enzymes involved in broad regulatory function, enzymes involved in amino acid synthesis, enzymes involved in nucleotide synthesis, enzymes involved in cofactor and vitamin synthesis, can be used for industrial biosynthesis.

The various metabolic pathways present in *Staphylococcus aureus* can be identified based on absolute nutritional requirements as well as by examining the various enzymes identified in Table 1-3 and SEQ ID NOS:1-5,191.

Of particular interest are polypeptides involved in the degradation of intermediary metabolites as well as non-macromolecular metabolism. Such enzymes include amylases, glucose oxidases, and catalase.

Proteolytic enzymes are another class of commercially important enzymes. Proteolytic enzymes find use in a number of industrial processes including the processing of flax and other vegetable fibers, in the extraction, clarification and depectinization of fruit juices, in the extraction of vegetables' oil and in the maceration of fruits and vegetables to give unicellular fruits. A detailed review of the proteolytic enzymes used in the food industry is provided in Rombouts *et al., Symbiosis* 21: 79 (1986) and Voragen *et al.* in BIOCATALYSTS IN AGRICULTURAL BIOTECHNOLOGY, Whitaker *et al.,* Eds., *American Chemical Society Symposium Series* 389: 93 (1989) .

The metabolism of sugars is an important aspect of the primary metabolism of *Staphylococcus aureus.* Enzymes involved in the degradation of sugars, such as, particularly, glucose, galactose, fructose and xylose, can be used in industrial fermentation. Some of the important sugar transforming enzymes, from a commercial viewpoint, include sugar isomerases such as glucose isomerase. Other metabolic enzymes have found commercial use such as glucose oxidases which produces ketogulonic acid (KGA). KGA is an intermediate in the commercial production of ascorbic acid using the Reichstein's procedure, as described in Krueger *et al., Biotechnology* 6(A), Rhine *et al.,* Eds., Verlag Press, Weinheim, Germany (1984).

Glucose oxidase (GOD) is commercially available and has been used in purified form as well as in an immobilized form for the deoxygenation of beer. See, for instance, Hartmeir *et al., Biotechnology Letters* 1: 21 (1979). The most important application of GOD is the industrial scale fermentation of gluconic acid. Market for gluconic acids which are used in the detergent, textile, leather, photographic, pharmaceutical, food, feed and concrete industry, as described, for example, in Bigelis *et al.,* beginning on page 357 in GENE MANIPULATIONS AND FUNGI; Benett *et al.,* Eds., Academic Press, New York (1985). In addition to industrial applications, GOD has found applications in medicine for

quantitative determination of glucose in body fluids recently in biotechnology for analyzing syrups from starch and cellulose hydrosylates. This application is described in Owusu *et al., Biochem. et Biophysica. Acta.* 872: 83 (1986), for instance.

The main sweetener used in the world today is sugar which comes from sugar beets and sugar cane. In the field of industrial enzymes, the glucose isomerase process shows the largest expansion in the market today. Initially, soluble enzymes were used and later immobilized enzymes were developed (Krueger *et al.*, Biotechnology, The Textbook of Industrial Microbiology, Sinauer Associated Incorporated, Sunderland, Massachusetts (1990)). Today, the use of glucose- produced high fructose syrups is by far the largest industrial business using immobilized enzymes. A review of the industrial use of these enzymes is provided by Jorgensen, Starch 40:307 (1988).

Proteinases, such as alkaline serine proteinases, are used as detergent additives and thus represent one of the largest volumes of microbial enzymes used in the industrial sector. Because of their industrial importance, there is a large body of published and unpublished information regarding the use of these enzymes in industrial processes. (See Faultman *et al.*, Acid Proteases Structure Function and Biology, Tang, J., ed., Plenum Press, New York (1977) and Godfrey *et al.*, Industrial Enzymes, MacMillan Publishers, Surrey, UK (1983) and Hepner *et al.*, Report Industrial Enzymes by 1990, Hel Hepner & Associates, London (1986)).

Another class of commercially usable proteins of the present invention are the microbial lipases, described by, for instance, Macrae *et al.*, Philosophical Transactions of the Chiral Society of London 310:227 (1985) and Poserke, Journal of the American Oil Chemist Society 61:1758 (1984). A major use of lipases is in the fat and oil industry for the production of neutral glycerides using lipase catalyzed inter-esterification of readily available triglycerides. Application of lipases include the use as a detergent additive to facilitate the removal of fats from fabrics in the course of the washing procedures.

The use of enzymes, and in particular microbial enzymes, as catalyst for key steps in the synthesis of complex organic molecules is gaining popularity at a great rate. One area of great interest is the preparation of chiral intermediates. Preparation of chiral intermediates is of interest to a wide range of synthetic chemists particularly those scientists involved with the preparation of new pharmaceuticals, agrochemicals, fragrances and flavors. (See Davies *et al.*, Recent Advances in the Generation of Chiral Intermediates Using Enzymes, CRC Press, Boca Raton, Florida (1990)). The following reactions catalyzed by enzymes are of interest to organic chemists:hydrolysis of carboxylic acid esters, phosphate esters, amides and nitriles, esterification reactions, trans-esterification reactions, synthesis of amides, reduction of alkanones and oxoalkanates, oxidation of alcohols to carbonyl compounds, oxidation of sulfides to sulfoxides, and carbon bond forming reactions such as the aldol reaction.

When considering the use of an enzyme encoded by one of the ORFs of the present invention for biotransformation and organic synthesis it is sometimes necessary to consider the respective advantages and disadvantages of using a microorganism as opposed to an isolated enzyme. Pros and cons of using a whole cell system on the one hand or an isolated partially purified enzyme on the other hand, has been described in detail by Bud *et al.*, Chemistry in Britain (1987), p. 127.

Amino transferases, enzymes involved in the biosynthesis and metabolism of amino acids, are useful in the catalytic production of amino acids. The advantages of using microbial based enzyme systems is that the amino transferase enzymes catalyze the stereo- selective synthesis of only L-amino acids and generally possess uniformly high catalytic rates. A description of the use of amino transferases for amino acid production is provided by Roselle-David, *Methods of Enzymology* 136:479 (1987).

Another category of useful proteins encoded by the ORFs of the present invention include enzymes involved in nucleic acid synthesis, repair, and recombination. A variety of commercially important enzymes have previously been isolated from members of *Staphylococcus aureus*. These include Sau3A and Sau96I.

2. Generation of Antibodies

As described here, the proteins of the present invention, as well as homologs thereof, can be used in a variety procedures and methods known in the art which are currently applied to other proteins. The proteins of the present invention can further be used to generate an antibody which selectively binds the protein. Such antibodies can be either monoclonal or polyclonal antibodies, as well fragments of these antibodies, and humanized forms.

The invention further provides antibodies which selectively bind to one of the proteins of the present invention and hybridomas which produce these antibodies. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing polyclonal and monoclonal antibodies as well as hybridomas capable of producing the desired antibody are well known in the art (Campbell, A. M., MONOCLONAL ANTIBODY TECHNOLOGY: LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY, Elsevier Science Publishers, Amsterdam, The Netherlands (1984); St. Groth *et al., J. Immunol. Methods* 35: 1-21 (1980), Kohler and Milstein, *Nature* 256: 495-497 (1975)), the trioma technique, the human B- cell hybridoma technique (Kozbor *et al., Immunology Today*

4: 72 (1983), pgs. 77-96 of Cole *et al.*, in MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc. (1985)).

Any animal (mouse, rabbit, *etc.*) which is known to produce antibodies can be immunized with the pseudogene polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or interperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of the protein encoded by the ORF of the present invention used for immunization will vary based on the animal which is immunized, the antigenicity of the peptide and the site of injection.

The protein which is used as an immunogen may be modified or administered in an adjuvant in order to increase the protein's antigenicity. Methods of increasing the antigenicity of a protein are well known in the art and include, but are not limited to coupling the antigen with a heterologous protein (such as globulin or galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells.

Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz *et al., Exp. Cell Res.* 175: 109-124 (1988)).

Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, A. M., Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1984)).

Techniques described for the production of single chain antibodies (U. S. Patent 4,946,778) can be adapted to produce single chain antibodies to proteins of the present invention.

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures.

The present invention further provides the above- described antibodies in detectably labelled form. Antibodies can be detectably labelled through the use of radioisotopes, affinity labels (such as biotin, avidin, *etc.*), enzymatic labels (such as horseradish peroxidase, alkaline phosphatase, *etc.*) fluorescent labels (such as FITC or rhodamine, *etc.*), paramagnetic atoms, *etc.* Procedures for accomplishing such labelling are well-known in the art, for example see Sternberger *et al.*, J. Histochem. Cytochem. 18:315 (1970); Bayer, E. A. *et al.*, Meth. Enzym. 62:308 (1979); Engval, E. *et al.*, Immunol. 109:129 (1972); Goding, J. W. J. Immunol. Meth. 13:215 (1976)).

The labeled antibodies of the present invention can be used for *in vitro, in vivo,* and in situ assays to identify cells or tissues in which a fragment of the *Staphylococcus aureus* genome is expressed.

The present invention further provides the above-described antibodies immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir, D. M. *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby, W. D. *et al.*, Meth. Enzym. 34 Academic Press, N. Y. (1974)). The immobilized antibodies of the present invention can be used for *in vitro, in vivo,* and in situ assays as well as for immunoaffinity purification of the proteins of the present invention.

3. Diagnostic Assays and Kits

The present invention further provides methods to identify the expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using one of the DFs,antigens or antibodies of the present invention.

In detail, such methods comprise incubating a test sample with one or more of the antibodies, or one or more of the DFs, or one or more antigens of the present invention and assaying for binding of the DFs, antigens or antibodies to components within the test sample.

Conditions for incubating a DF, antigen or antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the DF or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the Dfs, antigens or antibodies of the present invention. Examples of such assays can be found in Chard, T., An Introduction to Radioimmunoassay and Related Techniques, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G. R. *et al.*, Techniques in Immunocytochemistry, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., Practice and Theory of Enzyme Immunoassays: Laboratory Techniques in Biochemistry; PCT publication WO95/32291, and Molecular Biology, Elsevier Science Publishers, Amsterdam, The Netherlands (1985), all of which are hereby incorporated herein by reference.

The test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based

on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention.

Specifically, the invention provides a compartmentalized kit to receive, in close confinement, one or more containers which comprises:(a) a first container comprising one of the Dfs, antigens or antibodies of the present invention; and (b) one or more other containers comprising one or more of the following:wash reagents, reagents capable of detecting presence of a bound DF, antigen or antibody.

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, *etc.*), and containers which contain the reagents used to detect the bound antibody, antigen or DF.

Types of detection reagents include labelled nucleic acid probes, labelled secondary antibodies, or in the alternative, if the primary antibody is labelled, the enzymatic, or antibody binding reagents which are capable of reacting with the labelled antibody. One skilled in the art will readily recognize that the disclosed Dfs, antigens and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

4. Screening Assay for Binding Agents

Using the isolated proteins of the present invention, the present invention further provides methods of obtaining and identifying agents which bind to a protein encoded by one of the ORFs of the present invention or to one of the fragments and the *Staphylococcus aureus* fragment and contigs herein described.

In general, such methods comprise steps of:

(a) contacting an agent with an isolated protein encoded by one of the ORFs of the present invention, or an isolated fragment of the Staphylococcus aureus genome; and

(b) determining whether the agent binds to said protein or said fragment.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention.

Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby *et al.*, Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W. H. Freeman, NY (1992), pp. 289-307, and Kaspczak *et al.*, Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control.

One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix by binding to DNA or RNA. Such agents can be based on the classic phosphodiester, ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods usually contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee *et al.*, Nucl. Acids Res. 6:3073 (1979); Cooney *et al.*, Science 241:456 (1988); and Dervan *et al.*, Science 251: 1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated

to be effective in model systems. Information contained in the sequences of the present invention can be used to design antisense and triple helix-forming oligonucleotides, and other DNA binding agents.

5. Pharmaceutical Compositions and Vaccines

The present invention further provides pharmaceutical agents which can be used to modulate the growth or pathogenicity of *Staphylococcus aureus,* or another related organism, *in vivo* or *in vitro.* As used herein, a "pharmaceutical agent" is defined as a composition of matter which can be formulated using known techniques to provide a pharmaceutical compositions. As used herein, the "pharmaceutical agents of the present invention" refers the pharmaceutical agents which are derived from the proteins encoded by the ORFs of the present invention or are agents which are identified using the herein described assays.

As used herein, a pharmaceutical agent is said to "modulate the growth or pathogenicity of *Staphylococcus aureus* or a related organism, *in vivo* or *in vitro,"* when the agent reduces the rate of growth, rate of division, or viability of the organism in question. The pharmaceutical agents of the present invention can modulate the growth or pathogenicity of an organism in many fashions, although an understanding of the underlying mechanism of action is not needed to practice the use of the pharmaceutical agents of the present invention. Some agents will modulate the growth or pathogenicity by binding to an important protein thus blocking the biological activity of the protein, while other agents may bind to a component of the outer surface of the organism blocking attachment or rendering the organism more prone to act the bodies nature immune system. Alternatively, the agent may comprise a protein encoded by one of the ORFs of the present invention and serve as a vaccine. The development and use of vaccines derived from membrane associated polypeptides are well known in the art. The inventors have identified particularly preferred immunogenic Staphylococcus aureus polypeptides for use as vaccines. Such immunogenic polypeptides are described above and summarized in Table 4, below.

As used herein, a "related organism" is a broad term which refers to any organism whose growth or pathogenicity can be modulated by one of the pharmaceutical agents of the present invention. In general, such an organism will contain a homolog of the protein which is the target of the pharmaceutical agent or the protein used as a vaccine. As such, related organisms do not need to be bacterial but may be fungal or viral pathogens.

The pharmaceutical agents and compositions of the present invention may be administered in a convenient manner, such as by the oral, topical, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. The pharmaceutical compositions are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, they are administered in an amount of at least about 1 mg/kg body weight and in most cases they will be administered in an amount not in excess of about 1 g/kg body weight per day. In most cases, the dosage is from about 0.1 mg/kg to about 10 g/kg body weight daily, taking into account the routes of administration, symptoms, *etc.*

The agents of the present invention can be used in native form or can be modified to form a chemical derivative. As used herein, a molecule is said to be a "chemical derivative" of another molecule when it contains additional chemical moieties not normally a part of the molecule. Such moieties may improve the molecule's solubility, absorption, biological half life, *etc.* The moieties may alternatively decrease the toxicity of the molecule, eliminate or attenuate any undesirable side effect of the molecule, *etc.* Moieties capable of mediating such effects are disclosed in, among other sources, REMINGTON'S PHARMACEUTICAL SCIENCES (1980) cited elsewhere herein.

For example, such moieties may change an immunological character of the functional derivative, such as affinity for a given antibody. Such changes in immunomodulation activity are measured by the appropriate assay, such as a competitive type immunoassay. Modifications of such protein properties as redox or thermal stability, biological half-life, hydrophobicity, susceptibility to proteolytic degradation or the tendency to aggregate with carriers or into multimers also may be effected in this way and can be assayed by methods well known to the skilled artisan.

The therapeutic effects of the agents of the present invention may be obtained by providing the agent to a patient by any suitable means (*e.g.,* inhalation, intravenously, intramuscularly, subcutaneously, enterally, or parenterally). It is preferred to administer the agent of the present invention so as to achieve an effective concentration within the blood or tissue in which the growth of the organism is to be controlled. To achieve an effective blood concentration, the preferred method is to administer the agent by injection. The administration may be by continuous infusion, or by single or multiple injections.

In providing a patient with one of the agents of the present invention, the dosage of the administered agent will vary depending upon such factors as the patient's age, weight, height, sex, general medical condition, previous medical history, *etc.* In general, it is desirable to provide the recipient with a dosage of agent which is in the range of from about 1 pg/kg to 10 mg/kg (body weight of patient), although a lower or higher dosage may be administered. The therapeutically effective dose can be lowered by using combinations of the agents of the present invention or another agent.

As used herein, two or more compounds or agents are said to be administered "in combination" with each other when either (1) the physiological effects of each compound, or (2) the serum concentrations of each compound can

be measured at the same time. The composition of the present invention can be administered concurrently with, prior to, or following the administration of the other agent.

The agents of the present invention are intended to be provided to recipient subjects in an amount sufficient to decrease the rate of growth (as defined above) of the target organism.

The administration of the agent(s) of the invention may be for either a "prophylactic" or "therapeutic" purpose. When provided prophylactically, the agent(s) are provided in advance of any symptoms indicative of the organisms growth. The prophylactic administration of the agent(s) serves to prevent, attenuate, or decrease the rate of onset of any subsequent infection. When provided therapeutically, the agent(s) are provided at (or shortly after) the onset of an indication of infection. The therapeutic administration of the compound(s) serves to attenuate the pathological symptoms of the infection and to increase the rate of recovery.

The agents of the present invention are administered to a subject, such as a mammal, or a patient, in a pharmaceutically acceptable form and in a therapeutically effective concentration. A composition is said to be "pharmacologically acceptable" if its administration can be tolerated by a recipient patient. Such an agent is said to be administered in a "therapeutically effective amount" if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient patient.

The agents of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby these materials, or their functional derivatives, are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, *e. g.,* human serum albumin, are described, for example, in REMINGTON'S PHARMACEUTICAL SCIENCES, 16th Ed., Osol, A., Ed., Mack Publishing, Easton PA (1980). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the agents of the present invention, together with a suitable amount of carrier vehicle.

Additional pharmaceutical methods may be employed to control the duration of action. Control release preparations may be achieved through the use of polymers to complex or absorb one or more of the agents of the present invention. The controlled delivery may be effectuated by a variety of well known techniques, including formulation with macromolecules such as, for example, polyesters, polyamino acids, polyvinyl, pyrrolidone, ethylenevinylacetate, methylcellulose, carboxymethylcellulose, or protamine, sulfate, adjusting the concentration of the macromolecules and the agent in the formulation, and by appropriate use of methods of incorporation, which can be manipulated to effectuate a desired time course of release. Another possible method to control the duration of action by controlled release preparations is to incorporate agents of the present invention into particles of a polymeric material such as polyesters, polyamino acids, hydrogels, poly(lactic acid) or ethylene vinylacetate copolymers. Alternatively, instead of incorporating these agents into polymeric particles, it is possible to entrap these materials in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization with, for example, hydroxymethylcellulose or gelatine-microcapsules and poly(methylmethacylate) microcapsules, respectively, or in colloidal drug delivery systems, for example, liposomes, albumin microspheres, microemulsions, nanoparticles, and nanocapsules or in macroemulsions. Such techniques are disclosed in REMINGTON'S PHARMACEUTICAL SCIENCES (1980).

The invention further provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

In addition, the agents of the present invention may be employed in conjunction with other therapeutic compounds.

6. Shot-Gun Approach to Megabase DNA Sequencing

The present invention further demonstrates that a large sequence can be sequenced using a random shotgun approach. This procedure, described in detail in the examples that follow, has eliminated the up front cost of isolating and ordering overlapping or contiguous subclones prior to the start of the sequencing protocols.

Certain aspects of the present invention are described in greater detail in the examples that follow. The examples are provided by way of illustration. Other aspects and embodiments of the present invention are contemplated by the inventors, as will be clear to those of skill in the art from reading the present disclosure.

ILLUSTRATIVE EXAMPLES

LIBRARIES AND SEQUENCING

1. Shotgun Sequencing Probability Analysis

The overall strategy for a shotgun approach to whole genome sequencing follows from the Lander and Waterman (Landerman and Waterman, Genomics 2: 231 (1988)) application of the equation for the Poisson distribution. According to this treatment, the probability, P_0, that any given base in a sequence of size L, in nucleotides, is not sequenced after a certain amount, n, in nucleotides, of random sequence has been determined can be calculated by the equation $P_0 = e^{-m}$, where m is L/n, the fold coverage." For instance, for a genome of 2.8 Mb, m=1 when 2.8 Mb of sequence has been randomly generated (1X coverage). At that point, $P_0 = e^{-1} = 0.37$. The probability that any given base has not been sequenced is the same as the probability that any region of the whole sequence L has not been determined and, therefore, is equivilent to the fraction of the whole sequence that has yet to be determined. Thus, at one-fold coverage, approximately 37% of a polynucleotide of size L, in nucleotides has not been sequenced. When 14 Mb of sequence has been generated, coverage is 5X for a .2.8 Mb and the unsequenced fraction drops to .0067 or 0.67%. 5X coverage of a 2.8 Mb sequence can be attained by sequencing approximately 17,000 random clones from both insert ends with an average sequence read length of 410 bp.

Similarly, the total gap length, G, is determined by the equation $G = Le^{-m}$, and the average gap size, g, follows the equation, g = L/n. Thus, 5X coverage leaves about 240 gaps averaging about 82 bp in size in a sequence of a polynucleotide 2.8 Mb long.

The treatment above is essentially that of Lander and Waterman, *Genomics* 2: 231 (1988).

2. Random Library Construction

In order to approximate the random model described above during actual sequencing, a nearly ideal library of cloned genomic fragments is required. The following library construction procedure was developed to achieve this end.

Staphylococcus aureus DNA was prepared by phenol extraction. A mixture containing 600 ug DNA in 3.3 ml of 300 mM sodium acetate, 10 mM Tris-HCl, 1 mM Na-EDTA, 30% glycerol was sonicated for 1 min. at 0°C in a Branson Model 450 Sonicator at the lowest energy setting using a 3 mm probe. The sonicated DNA was ethanol precipitated and redissolved in 500 ul TE buffer.

To create blunt-ends, a 100 ul aliquot of the resuspended DNA was digested with 5 units of BAL31 nuclease (New England BioLabs) for 10 min at 30°C in 200 ul BAL31 buffer . The digested DNA was phenol-extracted, ethanol-precipitated, redissolved in 100 ul TE buffer, and then size-fractionated by electrophoresis through a 1.0% low melting temperature agarose gel. The section containing DNA fragments 1.6-2.0 kb in size was excised from the gel, and the LGT agarose was melted and the resulting solution was extracted with phenol to separate the agarose from the DNA. DNA was ethanol precipitated and redissolved in 20 ul of TE buffer for ligation to vector.

A two-step ligation procedure was used to produce a plasmid library with 97% inserts, of which >99% were single inserts. The first ligation mixture (50 ul) contained 2 ug of DNA fragments, 2 ug pUC18 DNA (Pharmacia) cut with SmaI and dephosphorylated with bacterial alkaline phosphatase, and 10 units of T4 ligase (GIBCO/BRL) and was incubated at 14°C for 4 hr. The ligation mixture then was phenol extracted and ethanol precipitated, and the precipitated DNA was dissolved in 20 ul TE buffer and electrophoresed on a 1.0% low melting agarose gel. Discrete bands in a ladder were visualized by ethidium bromide-staining and UV illumination and identified by size as insert (i), vector (v), v+i, v+2i, v+3i, *etc.* The portion of the gel containing v+i DNA was excised and the v+i DNA was recovered and resuspended into 20 ul TE. The v+i DNA then was blunt-ended by T4 polymerase treatment for 5 min. at 37° C in a reaction mixture (50 ul) containing the v+i linears, 500 uM each of the 4 dNTPs, and 9 units of T4 polymerase (New England BioLabs), under recommended buffer conditions. After phenol extraction and ethanol precipitation the repaired v+i linears were dissolved in 20 ul TE. The final ligation to produce circles was carried out in a 50 ul reaction containing 5 ul of v+i linears and 5 units of T4 ligase at 14°C overnight. After 10 min. at 70°C the following day, the reaction mixture was stored at -20°C.

This two-stage procedure resulted in a molecularly random collection of single-insert plasmid recombinants with minimal contamination from double-insert chimeras (<1%) or free vector (<3%).

Since deviation from randomness can arise from propagation the DNA in the host, *E.coli* host cells deficient in all recombination and restriction functions (A. Greener, Strategies 3 (1):5 (1990)) were used to prevent rearrangements, deletions, and loss of clones by restriction. Furthermore, transformed cells were plated directly on antibiotic diffusion plates to avoid the usual broth recovery phase which allows multiplication and selection of the most rapidly growing cells.

Plating was carried out as follows. A 100 ul aliquot of Epicurian Coli SURE II Supercompetent Cells (Stratagene 200152) was thawed on ice and transferred to a chilled Falcon 2059 tube on ice. A 1.7 ul aliquot of 1.42 M beta-

mercaptoethanol was added to the aliquot of cells to a final concentration of 25 mM. Cells were incubated on ice for 10 min. A 1 ul aliquot of the final ligation was added to the cells and incubated on ice for 30 min. The cells were heat pulsed for 30 sec. at 42° C and placed back on ice for 2 min. The outgrowth period in liquid culture was eliminated from this protocol in order to minimize the preferential growth of any given transformed cell. Instead the transformation mixture was plated directly on a nutrient rich SOB plate containing a 5 ml bottom layer of SOB agar (5% SOB agar: 20 g tryptone, 5 g yeast extract, 0.5 g NaCl, 1.5% Difco Agar per liter of media). The 5 ml bottom layer is supplemented with 0.4 ml of 50 mg/ml ampicillin per 100 ml SOB agar. The 15 ml top layer of SOB agar is supplemented with 1 ml X-Gal (2%), 1 ml $MgCl_2$ (1 M), and 1 ml $MgSO_4$/100 ml SOB agar. The 15 ml top layer was poured just prior to plating. Our titer was approximately 100 colonies/10 ul aliquot of transformation.

All colonies were picked for template preparation regardless of size. Thus, only clones lost due to "poison" DNA or deleterious gene products would be deleted from the library, resulting in a slight increase in gap number over that expected.

3. Random DNA Sequencing

High quality double stranded DNA plasmid templates were prepared using an alkaline lysis method developed in collaboration with 5Prime → 3Prime Inc. (Boulder, CO). Plasmid preparation was performed in a 96-well format for all stages of DNA preparation from bacterial growth through final DNA purification. Average template concentration was determined by running 25% of the samples on an agarose gel. DNA concentrations were not adjusted.

Templates were also prepared from a *Staphylococcus aureus* lambda genomic library. An unamplified library was constructed in Lambda DASH II vector (Stratagene). *Staphylococcus aureus* DNA (> 100 kb) was partially digested in a reaction mixture (200 ul) containing 50 ug DNA, 1X Sau3AI buffer, 20 units Sau3AI for 6 min. at 23 C. The digested DNA was phenol-extracted and centrifuges over a 10- 40% sucroce gradient. Fractions containing genomic DNA of 15-25 kb were recovered by precipitation . One ul of fragments was used with 1 ul of DASHII vector (Stratagene) in the recommended ligation reaction. One ul of the ligation mixture was used per packaging reaction following the recommended protocol with the Gigapack II XL Packaging Extract Phage were plated directly without amplification from the packaging mixture (after dilution with 500 ul of recommended SM buffer and chloroform treatment). Yield was about $2.5x10^9$ pfu/ul.

An amplified library was prepared from the primary packaging mixture according to the manufactureer's protocol. The amplified library is stored frozen in 7% dimethylsulfoxide. The phage titer is approximately $1x10^9$ pfu/ml.

Mini-liquid lysates (0.1ul) are prepared from randomly selected plaques and template is prepared by long range PCR. Samples are PCR amplified using modified T3 and T7 primers, and Elongase Supermix (LTI).

Sequencing reactions are carried out on plasmid templates using a combination of two workstations (BIOMEK 1000 and Hamilton Microlab 2200) and the Perkin-Elmer 9600 thermocycler with Applied Biosystems PRISM Ready Reaction Dye Primer Cycle Sequencing Kits for the M13 forward (M13-21) and the M13 reverse (M13RP1) primers. Dye terminator sequencing reactions are carried out on the lambda templates on a Perkin-Elmer 9600 Thermocycler using the Applied Biosystems Ready Reaction Dye Terminator Cycle Sequencing kits. Modified T7 and T3 primers are used to sequence the ends of the inserts from the Lambda DASH II library. Sequencing reactions are on a combination of AB 373 DNA Sequencers and ABI 377 DNA sequencers. All of the dye terminator sequencing reactions are analyzed using the 2X 9 hour module on the AB 377. Dye primer reactions are analyzed on a combination of ABI 373 and ABI 377 DNA sequencers. The overall sequencing success rate very approximately is about 85% for M13-21 and M13RP1 sequences and 65% for dye-terminator reactions. The average usable read length is 485 bp for M13-21 sequences, 445bp for M13RP1 sequences, and 375 bp for dye-terminator reactions.

4. Protocol for Automated Cycle Sequencing

The sequencing was carried out using Hamilton Microstation 2200, Perkin Elmer 9600 thermocyclers, ABI 373 and ABI 377 Automated DNA Sequencers. The Hamilton combines pre-aliquoted templates and reaction mixes consisting of deoxy- and dideoxynucleotides, the thermostable Taq DNA polymerase, fluorescently-labelled sequencing primers, and reaction buffer. Reaction mixes and templates were combined in the wells of a 96-well thermocycling plate and transferred to the Perkin Elmer 9600 thermocycler. Thirty consecutive cycles of linear amplification (i.e.., one primer synthesis) steps were performed including denaturation, annealing of primer and template, and extension; i.e., DNA synthesis. A heated lid with rubber gaskets on the thermocycling plate prevents evaporation without the need for an oil overlay.

Two sequencing protocols were used: one for dye-labelled primers and a second for dye-labelled dideoxy chain terminators. The shotgun sequencing involves use of four dye-labelled sequencing primers, one for each of the four terminator nucleotide. Each dye-primer was labelled with a different fluorescent dye, permitting the four individual reactions to be combined into one lane of the 373 or 377 DNA Sequencer for electrophoresis, detection, and base-

calling. ABI currently supplies premixed reaction mixes in bulk packages containing all the necessary non-template reagents for sequencing. Sequencing can be done with both plasmid and PCR-generated templates with both dye-primers and dye- terminators with approximately equal fidelity, although plasmid templates generally give longer usable sequences.

Thirty-two reactions were loaded per ABI 373 Sequencer each day and 96 samples can be loaded on an ABI 377 per day. Electrophoresis was run overnight (ABI 373) or for 2 1/2 hours (ABI 377) following the manufacturer's protocols. Following electrophoresis and fluorescence detection, the ABI 373 or ABI 377 performs automatic lane tracking and base-calling. The lane-tracking was confirmed visually. Each sequence electropherogram (or fluorescence lane trace) was inspected visually and assessed for quality. Trailing sequences of low quality were removed and the sequence itself was loaded via software to a Sybase database (archived daily to 8mm tape). Leading vector polylinker sequence was removed automatically by a software program. Average edited lengths of sequences from the standard ABI 373 or ABI 377 were around 400 bp and depend mostly on the quality of the template used for the sequencing reaction.

INFORMATICS

1. Data Management

A number of information management systems for a large-scale sequencing lab have been developed. (For review see, for instance, Kerlavage *et al., Proceedings of the Twenty-Sixth Annual Hawaii International Conference on System Sciences,* IEEE Computer Society Press, Washington D. C., 585 (1993)) The system used to collect and assemble the sequence data was developed using the Sybase relational database management system and was designed to automate data flow whereever possible and to reduce user error. The database stores and correlates all information collected during the entire operation from template preparation to final analysis of the genome. Because the raw output of the ABI 373 Sequencers was based on a Macintosh platform and the data management system chosen was based on a Unix platform, it was necessary to design and implement a variety of multi- user, client-server applications which allow the raw data as well as analysis results to flow seamlessly into the database with a minimum of user effort.

2. Assembly

An assembly engine (TIGR Assembler) developed for the rapid and accurate assembly of thousands of sequence fragments was enployed to generate contigs. The TIGR assembler simultaneously clusters and assembles fragments of the genome. In order to obtain the speed necessary to assemble more than 10^4 fragments, the algorithm builds a hash table of 12 bp oligonucleotide subsequences to generate a list of potential sequence fragment overlaps. The number of potential overlaps for each fragment determines which fragments are likely to fall into repetitive elements. Beginning with a single seed sequence fragment, TIGR Assembler extends the current contig by attempting to add the best matching fragment based on oligonucleotide content. The contig and candidate fragment are aligned using a modified version of the Smith-Waterman algorithm which provides for optimal gapped alignments (Waterman, M. S., *Methods in Enzymology* 164: 765 (1988)). The contig is extended by the fragment only if strict criteria for the quality of the match are met. The match criteria include the minimum length of overlap, the maximum length of an unmatched end, and the minimum percentage match. These criteria are automatically lowered by the algorithm in regions of minimal coverage and raised in regions with a possible repetitive element. The number of potential overlaps for each fragment determines which fragments are likely to fall into repetitive elements. Fragments representing the boundaries of repetitive elements and potentially chimeric fragments are often rejected based on partial mismatches at the ends of alignments and excluded from the current contig. TIGR Assembler is designed to take advantage of clone size information coupled with sequencing from both ends of each template. It enforces the constraint that sequence fragments from two ends of the same template point toward one another in the contig and are located within a certain ranged of base pairs (definable for each clone based on the known clone size range for a given library).

3. Identifying Genes

The predicted coding regions of the *Staphylococcus aureus* genome were initially defined with the program zorf, which finds ORFs of a minimum length. The predicted coding region sequences were used in searches against a database of all *Staphylococcus aureus* nucleotide sequences from GenBank (release 92.0), using the BLASTN search method to identify overlaps of 50 or more nucleotides with at least a 95% identity. Those ORFs with nucleotide sequence matches are shown in Table 1. The ORFs without such matches were translated to protein sequences and and compared to a non-redundant database of known proteins generated by combining the Swiss-prot, PIR and GenPept databases. ORFs of at least 80 amino acids that matched a database protein with BLASTP probability less than or equal to 0.01 are shown in Table 2. The table also lists assigned functions based on the closest match in the databases.

ORFs of at least 120 amino acids that did not match protein or nucleotide sequences in the databases at these levels are shown in Table 3.

ILLUSTRATIVE APPLICATIONS

1. Production of an Antibody to a *Staphylococcus aureus* Protein

Substantially pure protein or polypeptide is isolated from the transfected or transformed cells using any one of the methods known in the art. The protein can also be produced in a recombinant prokaryotic expression system, such as *E. coli*, or can by chemically synthesized. Concentration of protein in the final preparation is adjusted, for example, by concentration on an Amicon filter device, to the level of a few micrograms/ml. Monoclonal or polyclonal antibody to the protein can then be prepared as follows.

2. Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256:495 (1975) or modifications of the methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein over a period of a few weeks. The mouse is then sacrificed, and the antibody producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells destroyed by growth of the system on selective media comprising aminopterin (HAT media). The successfully fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as originally described by Engvall, E., Meth. Enzymol. 70:419 (1980), and modified methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. *et al.* Basic Methods in Molecular Biology Elsevier, New York. Section 21-2 (1989).

3. Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogenous epitopes of a single protein can be prepared by immunizing suitable animals with the expressed protein described above, which can be unmodified or modified to enhance immunogenicity. Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than other and may require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigenadministered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. *et al.*, J. Clin. Endocrinol. Metab. 33:988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. *et al.*, Chap. 19 in:Handbook of Experimental Immunology, Wier, D., ed, Blackwell (1973). Plateau concentration of antibody is usually in the range of 0. 1 to 0. 2 mg/ml of serum (about 12M). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in:Manual of Clinical Immunology, second edition, Rose and Friedman, eds., Amer. Soc. For Microbiology, Washington, D. C. (1980)

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi- quantitatively or qualitatively to identify the presence of antigen in a biological sample. In addition, they are useful in various animal models of Staphylococcal disease known to those of skill in the art as a means of evaluating the protein used to make the antibody as a potential vaccine target or as a means of evaluating the antibody as a potential immunothereapeutic reagent.

3. Preparation of PCR Primers and Amplification of DNA

Various fragments of the *Staphylococcus aureus* genome, such as those of Tables 1-3 and SEQ ID NOS:1-5,191 can be used, in accordance with the present invention, to prepare PCR primers for a variety of uses. The PCR primers are preferably at least 15 bases, and more preferably at least 18 bases in length. When selecting a primer sequence, it is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approxi-

mately the same. The PCR primers and amplified DNA of this Example find use in the Examples that follow.

4. Gene expression from DNA Sequences Corresponding to ORFs

A fragment of the *Staphylococcus aureus* genome provided in Tables 1-3 is introduced into an expression vector using conventional technology. Techniques to transfer cloned sequences into expression vectors that direct protein translation in mammalian, yeast, insect or bacterial expression systems are well known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon context and codon pairing of the sequence may be optimized for the particular expression organism, as explained by Hatfield *et al.*, U. S. Patent No. 5,082,767, incorporated herein by this reference.

The following is provided as one exemplary method to generate polypeptide(s) from cloned ORFs of the *Staphylococcus aureus* genome fragment. Bacterial ORFs generally lack a poly A addition signal. The addition signal sequence can be added to the construct by, for example, splicing out the poly A addition sequence from pSG5 (Stratagene) using BglI and SalI restriction endonuclease enzymes and incorporating it into the mammalian expression vector pXT1 (Stratagene) for use in eukaryotic expression systems. pXT1 contains the LTRs and a portion of the gag gene of Moloney Murine Leukemia Virus. The positions of theLTRs in the construct allow efficient stable transfection. The vector includes the Herpes Simplex thymidine kinase promoter and the selectable neomycin gene. The *Staphylococcus aureus* DNA is obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the *Staphylococcus aureus* DNA and containing restriction endonuclease sequences for PstI incorporated into the 5' primer and BglII at the 5' end of the corresponding *Staphylococcus aureus* DNA 3' primer, taking care to ensure that the *Staphylococcus aureus* DNA is positioned such that its followed with the poly A addition sequence. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with BglII, purified and ligated to pXT1, now containing a poly A addition sequence and digested BglII.

The ligated product is transfected into mouse NIH 3T3 cells using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600 ug/ml G418 (Sigma, St. Louis, Missouri). The protein is preferably released into the supernatant. However if the protein has membrane binding domains, the protein may additionally be retained within the cell or expression may be restricted to the cell surface. Since it may be necessary to purify and locate the transfected product, synthetic 15-mer peptides synthesized from the predicted *Staphylococcus aureus* DNA sequence are injected into mice to generate antibody to the polypeptide encoded by the *Staphylococcus aureus* DNA.

Alternativly and if antibody production is not possible, the *Staphylococcus aureus* DNA sequence is additionally incorporated into eukaryotic expression vectors and expressed as, for example, a globin fusion. Antibody to the globin moiety then is used to purify the chimeric protein. Corresponding protease cleavage sites are engineered between the globin moiety and the polypeptide encoded by the *Staphylococcus aureus* DNA so that the latter may be freed from the formed by simple protease digestion. One useful expression vector for generating globin chimerics is pSG5 (Stratagene). This vector encodes a rabbit globin. Intron II of the rabbit globin gene facilitates splicing of the expressed transcript, and the polyadenylation signal incorporated into the construct increases the level of expression. These techniques are well known to those skilled in the art of molecular biology. Standard methods are published in methods texts such as Davis *et al.*, cited elsewhere herein, and many of the methods are available from the technical assistance representatives from Stratagene, Life Technologies, Inc., or Promega. Polypeptides of the invention also may be produced using *in vitro* translation systems such as *in vitro* ExpressTM Translation Kit (Stratagene).

While the present invention has been described in some detail for purposes of clarity and understanding, one skilled in the art will appreciate that various changes in form and detail can be made without departing from the true scope of the invention.

All patents, patent applications and publications referred to above are hereby incorporated by reference.

His Lys Ala
1025

(2) INFORMATION FOR SEQ ID NO:5255:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 155 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5255:

```
Gly Glu Lys Cys Met Phe Leu Ala Trp Asn Glu Ile Arg Arg Asn Lys
1               5                  10                  15

Leu Lys Phe Gly Leu Ile Ile Gly Val Leu Thr Met Ile Ser Tyr Leu
            20                  25                  30

Leu Phe Leu Leu Ser Gly Leu Ala Asn Gly Leu Ile Asn Met Asn Lys
        35                  40                  45

Glu Gly Ile Asp Lys Trp Gln Ala Asp Ala Ile Val Leu Asn Lys Asp
    50                  55                  60

Ala Asn Gln Thr Val Gln Gln Ser Val Phe Asn Lys Lys Asp Ile Glu
65                  70                  75                  80

Asn Lys Tyr Lys Lys Gln Ala Thr Leu Lys Gln Thr Gly Glu Ile Val
                85                  90                  95

Ser Asn Gly His Gln Lys Asp Asn Val Leu Val Phe Gly Val Glu Lys
            100                 105                 110

Ser Ser Phe Leu Val Pro Ser Leu Ile Glu Gly His Lys Ala Thr Lys
        115                 120                 125

Asp Asn Glu Val Leu Ala Asp Glu Thr Leu Lys Asn Lys Gly Leu Lys
    130                 135                 140

Leu Gly Asp Thr Leu Ser Leu Ser Xaa Xaa Arg
145                 150                 155
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Claims

1. Computer readable medium having recorded thereon a nucleotide sequence of the Staphylococcus aureus genome as depicted in SEQ ID NOS:1-5,191, a representative fragment thereof or a nucleotide sequence at least 95 % identical to a nucleotide sequence depicted in SEQ ID NOS:1-5,191.

2. Computer readable medium having recorded thereon any one of the fragments of SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a degenerate variant thereof.

3. The computer readable medium of claim 1, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

4. The computer readable medium of claim 3, wherein said medium is selected from the group consisting of a floppy disc, a hard disc, random access memory (RAM), read only memory (ROM), and CD-ROM.

5. A computer-based system for identifying fragments of the *Staphylococcus aureus* genome of commercial importance comprising the following elements:

(a) a data storage means comprising the nucleotide sequence of SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS: 1-5,191;
(b) search means for comparing a target sequence to the nucleotide sequence of the data storage means of step (a) to identify homologous sequence(s), and
(c) retrieval means for obtaining said homologous sequence(s) of step (b).

6. A method for identifying commercially important nucleic acid fragments of the *Staphylococcus aureus* genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS: 1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to a nucleotide sequence of SEQ ID NOS:1-5,191 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence is not randomly selected.

7. A method for identifying an expression modulating fragment of *Staphylococcus aureus* genome comprising the step of comparing a database comprising the nucleotide sequences depicted in SEQ ID NOS:1-5,191, a representative fragment thereof, or a nucleotide sequence at least 95% identical to the nucleotide sequence of SEQ ID NOS:1-5,191 with a target sequence to obtain a nucleic acid molecule comprised of a complementary nucleotide sequence to said target sequence, wherein said target sequence comprises sequences known to regulate gene expression.

8. A protein-encoding nucleic acid fragment of the Staphylococcus aureus genome,
wherein said fragment comprises the nucleotide sequence of any one of the fragments of SEQ ID NOS: 1-5,191 depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence, or a degenerate variant of any of the aforementioned sequences.

9. The nucleic acid fragment of claim 8 which is DNA.

10. The nucleic acid fragment of claim 8 which is RNA.

11. A vector comprising a fragment of claim 8.

12. A fragment of the Staphylococcus aureus genome, wherein said fragment modulates the expression of an operably liked open reading frame, wherein said fragment consists of the nucleotide sequence from about 10 to 200 bases in length which is 5' to any one of the open reading frames depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence or a degenerate variant of any of the aforementioned sequences.

13. A vector comprising a fragment of claim 12.

14. A organism which has been altered to contain any one of the fragments of the Staphylococcus aureus genome of claim 8.

15. A method for producing a polypeptide in a host cell comprising the steps of:

(a) incubating an organism of claim 14 under conditions where said fragment is expressed to produce said protein, and
(b) isolating said protein.

16. An organism which has been altered to contain any one of the fragments of the Staphylococcus aureus genome

of claim 12.

17. A method for regulating the expression of a nucleic acid molecule comprising the step of covalently attaching to said nucleic acid molecule a nucleic acid molecule consisting of the nucleotide sequence from about 30 to 300 bases 5' to any one of the fragments of the Staphylococcus aureus genome depicted in Seq ID Nos:1-5,191 and Tables 2 and 3 or a nucleotide sequence at least 95% identical to such a nucleotide sequence or a degenerate variant of any of the aforementioned sequences.

18. A nucleic acid molecule being a homolog of any of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising the steps of:

(a) screening a genomic DNA library using as a probe a target sequence defined by any of SEQ ID NOS: 1-5,191 and Tables 2 and 3, including fragments thereof;
(b) identifying members of said library which contain sequences that hybridize to said target sequence;
(c) isolating the nucleic acid molecules from said members identified in step (b).

19. A DNA molecule being a homolog of any one of the fragments of the Staphylococcus aureus genome of SEQ ID NOS:1-5,191 and Tables 2 and 3, wherein said nucleic acid molecule is produced by a process comprising the steps of:

(a) isolating mRNA, DNA, or cDNA produced from an organism;
(b) amplifying nucleic acid molecules whose nucleotide sequence is homologous to amplification primers derived from said fragment of said *Staphylococcus aureus* genome to prime said amplification;
(c) isolating said amplified sequences produced in step (b).

20. A polypeptide encoded by a fragment of claim 8.

21. An antibody which selectively binds to any one of the polypeptides of claim 20.

22. A kit for analyzing samples for the presence of polynucleotides derived from Staphylococcus aureus, comprising at least one polynucleotide containing a nucleotide sequence of any one of the fragments SEQ ID NOS:1-5,191 depicted in Tables 2 and 3 or a nucleotide sequence at least 95% identical thereto or a degenerate variant of any of the aforementioned sequences, that will hybridize to a staphylococcus aureus polynucleotide under stringent hybridization conditions, and a suitable container.

23. A Staphylococcus aureus polypeptide comprising an amino acid sequence identical to an amino acid sequence selected from the group consisting of SEQ ID NOS:5,192 to 5,255 or comprising an amino acid sequence having at least 95% identity to such a sequence.

24. A Staphylococcus aureus polypeptide antigen comprising at least one epitope derived from a Staphylococcus aureus polypeptide selected from the group consisting of SEQ ID NOS:5,192 to 5,255.

25. A polypeptide comprising at least one epitope encoded by a Staphylococcus aureus amino acid sequence selected from the group consisting of the epitopic sequences listed in Table 4.

26. The polypeptide of claim 24 or 26, wherein said polypeptide is fixed to a solid phase.

27. A diagnostic kit for detecting Staphylococcus aureus infection comprising

(a) an isolated polypeptide antigen of claim 24, and
(b) means for detecting the binding of an antibody contained in a biological fluid to said antigen.

28. A vaccine composition comprising a polypeptide of claim 24 present in a pharmaceutically acceptable carrier.

29. A method of vaccinating an individual against Staphylococcus aureus infection comprising, administering to an individual the vaccine composition of claim 28.

Figure 1

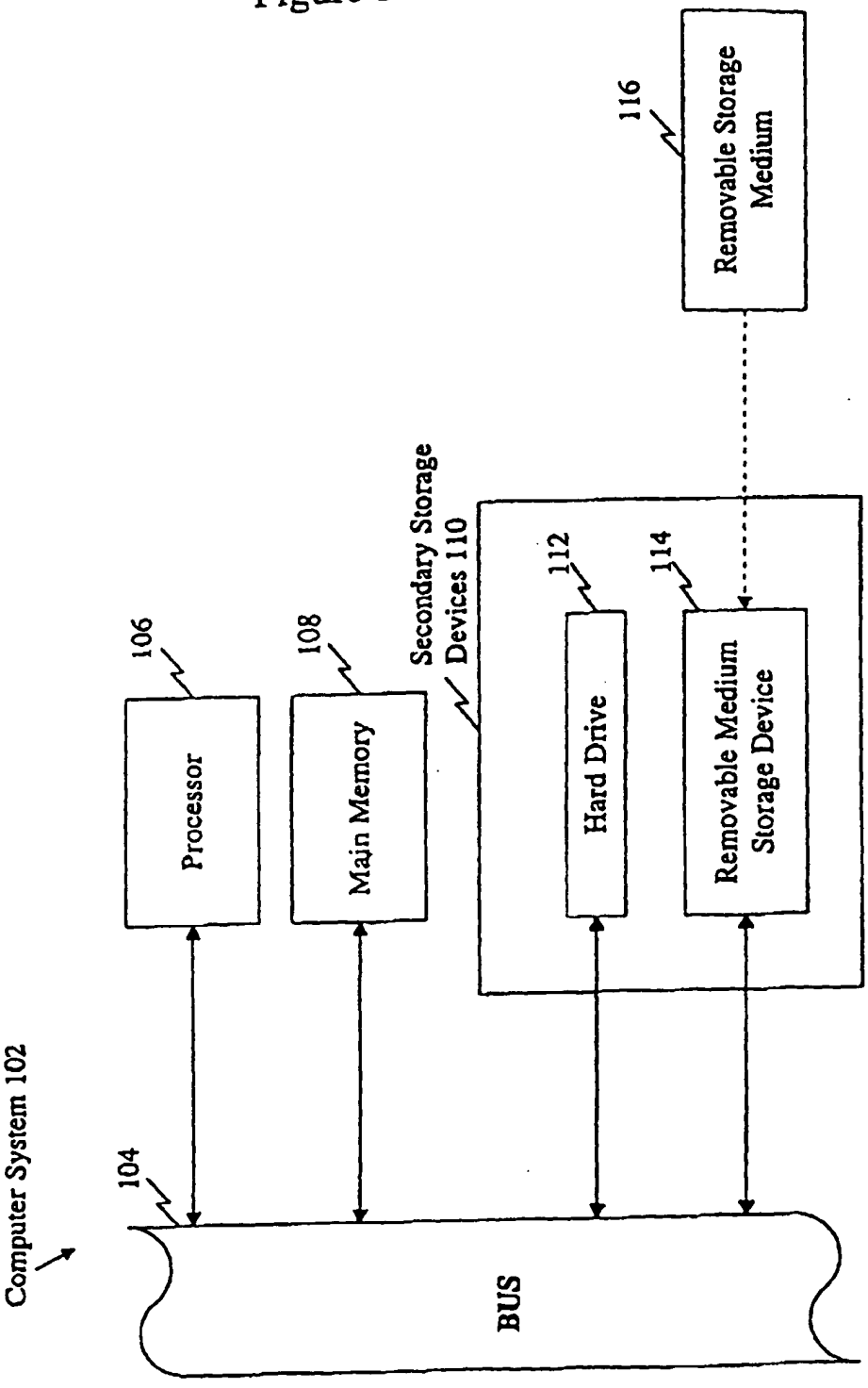
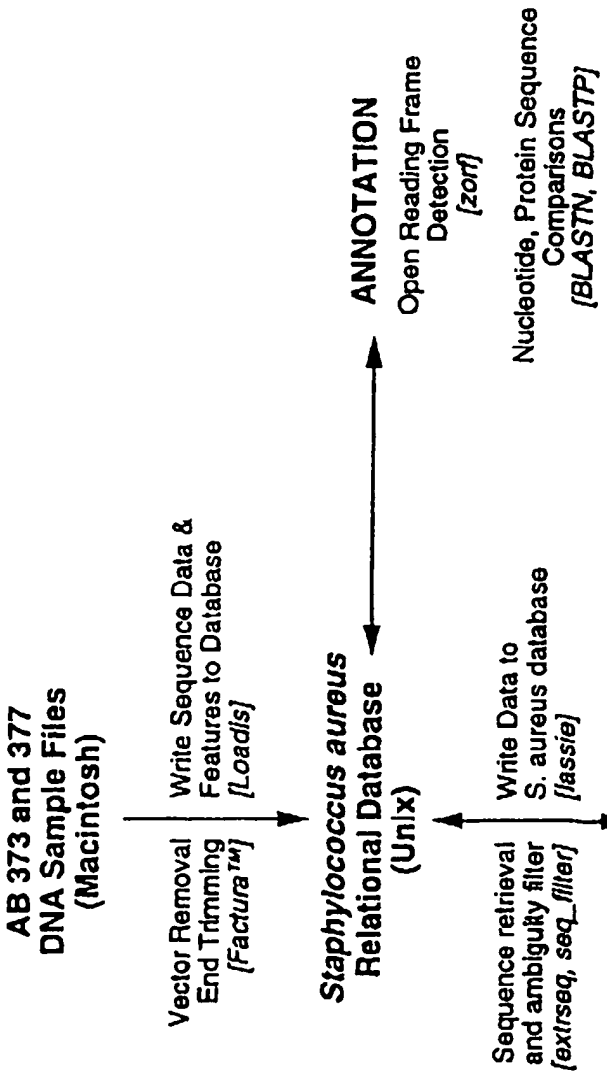

Computer System 102
104
BUS
Processor
106
Main Memory
108
Secondary Storage Devices 110
Hard Drive
112
Removable Medium Storage Device
114
Removable Storage Medium
116

Figure 2


AB 373 and 377
DNA Sample Files
(Macintosh)
Vector Removal
End Trimming
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Write Sequence Data &
Features to Database
[Loadis]
Staphylococcus aureus
Relational Database
(Unix)
Sequence retrieval
and ambiguity filter
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Write Data to
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ANNOTATION
Open Reading Frame
Detection
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Nucleotide, Protein Sequence
Comparisons
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Rapid Assembly
and Ordering of
Thousands of Sequences
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